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THE BETA GLUCURONIDASE CONTENT OF HUMAN LEUKOCYTES IN HEALTH AND IN DISEASE

JAMES H. FOLLETTE, M.D., WILLIAM N. VALENTINE, M.D., AND JOHN S. LAWRENCE, M.D.
LOS ANGELES, CALIF.

INTRODUCTION

HISTORICAL data pertaining to β-glucuronidase have been adequately reviewed by Fishman and associates[1] and by Rossiter and Wong.[2] Marked interest in β-glucuronidase has been prompted by the discovery that high β-glucuronidase activity is present in primary malignant neoplasms of the breast, uterus, ovary, stomach, colon, and in their metastases to other organs and to lymph nodes.[3-7] Of equal importance has been the observation that serum β-glucuronidase activity is related to estrogen metabolism.[6, 8, 9, 10]

The buffy coat of human blood was studied by Anlyan and Fishman[3] and by Fishman, Springer and Brunetti,[11] who found that a high percentage of the β-glucuronidase activity was present in the leukocytes. Negligible activity was noted in the platelets and erythrocytes. While elevations in total numbers of leukocytes were associated with increased enzymatic activity, this increase could not be more specifically related to either the numbers of lymphocytes or polymorphonuclear leukocytes.

Rossiter and Wong[12] extended Fishman's work on leukocytes, obtaining mixed white blood cell suspensions from normal human subjects by centrifuging in a constricted centrifuge tube[13] or by flotation on a solution of gum acacia.[14] Values were expressed in β-glucuronidase units per 10^{10} cells (one β-glucuronidase unit being the amount of enzyme that liberated one microgram of phenolphthalein per hour from the substrate, phenolphthalein-mono-β-glucuronide). By statistical methods, the enzyme was shown to be present in both the polymorphonuclear leukocyte and lymphocyte, the ratio being approximately 5:4. In 1950, Anlyan, Gamble, and Hoster[15] reported on the β-glucuronidase activity of the separated buffy coat of blood in patients with Hodgkin's disease, myelocytic leukemia, monocytic leukemia, lymphocytic leukemia, and in a variety of miscellaneous conditions. Consistent variations from normal were found in patients with chronic lymphocytic leukemia and in chronic and acute myelocytic leukemia. These workers related enzyme activity to wet weight of leukocytes.

This report summarizes recent studies on the β-glucuronidase activity of separated leukocytes from normal subjects and from subjects with various types of leukemia, other hematologic disorders, and miscellaneous disease states.

This investigation was supported by a research grant Number 1069 (C) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

From the Department of Medicine, School of Medicine, University of California Medical Center.

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METHODS

The method used in separating leukocytes from the erythrocytes of whole blood has previously been described.[16, 17] This essentially consists of mixing 20 ml. of venous blood with a freshly prepared solution of bovine fibrinogen containing citrate (Fraction I, Armour), so that the final solution results in a packed cell volume of approximately 30 volumes per cent. The leukocyte count per cubic millimeter of the sample is determined in duplicate and the results averaged. Smears are made from which two hundred cells are counted differentially.

After allowing the red cells in the fibrinogen mixture to sediment over a twenty-minute period, the supernatant plasma suspension is aspirated. This contains the leukocytes and platelets, and is free from erythrocytes for the most part. Centrifugation of this suspension at 1,200 r.p.m. for five minutes is then carried out. The supernatant is removed and discarded. The cells are washed once by resuspending in saline and centrifuging. They are then resuspended in saline so that their final concentration ranges from 5,000 to 15,000 cells per cubic millimeter, cell counts being done in triplicate on all suspensions. It was not considered necessary to separate the platelets from the suspension quantitatively in view of previous observations[11] that platelets possess no β-glucuronidase activity.

β-glucuronidase determinations are carried out by the method of Fishman and coworkers[11, 19] using phenolphthalein-mono-β-glucuronide as a substrate. Since saponin, bile salts, and alkyl sulfate detergents have been found to liberate β-glucuronidase from leukocytes,[2, 18] saponin is included in the reaction mixture. The following is added to duplicate centrifuge tubes:

Leukocytes in saline	0.5 ml.
Acetate buffer 0.1M, pH 4.5	1.0 ml.
Phenolphthalein-mono-β-glucuronide 0.01M	0.1 ml.
Saponin 0.5 per cent	0.4 ml.

Incubation is carried out for three hours at 38° C. in a water bath. The reaction is stopped by adding 1.0 ml. of 10 per cent trichloracetic acid.

Suitable blank and standard tubes are treated similarly, except that in the former the cells are added after the addition of the trichloracetic acid, and in the latter the phenolphthalein-mono-β-glucuronide is added after the trichloracetic acid. The standard contains 40 μg of phenolphthalein in all determinations. The remainder of the procedure is carried out as described by Fishman and his associates.[11] The final solutions are read in a Beckman Model B spectrophotometer at 540 mμ wave length using large Coleman cuvettes and a final volume of 10 milliliters. Values obtained are calculated in terms of milligrams of phenolphthalein liberated per hour per 10^{10} leukocytes. The amount of glucuronidase activity per milliliter of blood is determined by relating the above values to the peripheral blood leukocyte count. The micrograms of phenolphthalein liberated from substrate per hour by the number of cells in the incubation mixture is computed as follows, when a 40 microgram standard and a three-hour incubation period are employed:

$$\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times \frac{40}{3} = \text{micrograms of phenolphthalein liberated per hour}$$

Glucuronidase activity is then expressed in terms of the number of *milligrams* of phenolphthalein which would be liberated per hour from substrate by 10^{10} leukocytes. This figure is readily obtained by multiplying the micrograms liberated per hour in the incubation mixture by the factor $\frac{10^{10}}{\text{total number of cells in incubation mixture}}$ and dividing by 1,000 for conversion to milligrams. *In the subsequent discussion, therefore, reference to β-glucuronidase activity will*

arbitrarily be expressed as a figure indicating the number of milligrams liberated from substrate per hour by 10^{10} leukocytes. Each milligram so liberated is synonymous with 1,000 β-glucuronidase units.

RESULTS

Fig. 1 indicates on a scattergram the values obtained in the leukocytes of normal subjects, and of subjects with subacute and chronic myelocytic leukemia, eosinophilia, neutrophilic leukocytosis, chronic lymphocytic leukemia, and lymphocytosis of nonleukemic origin. As can be seen, the normal values fall within a relatively narrow range. The widest variations from normal were observed in the lymphocytic leukemias, in which very low concentrations of

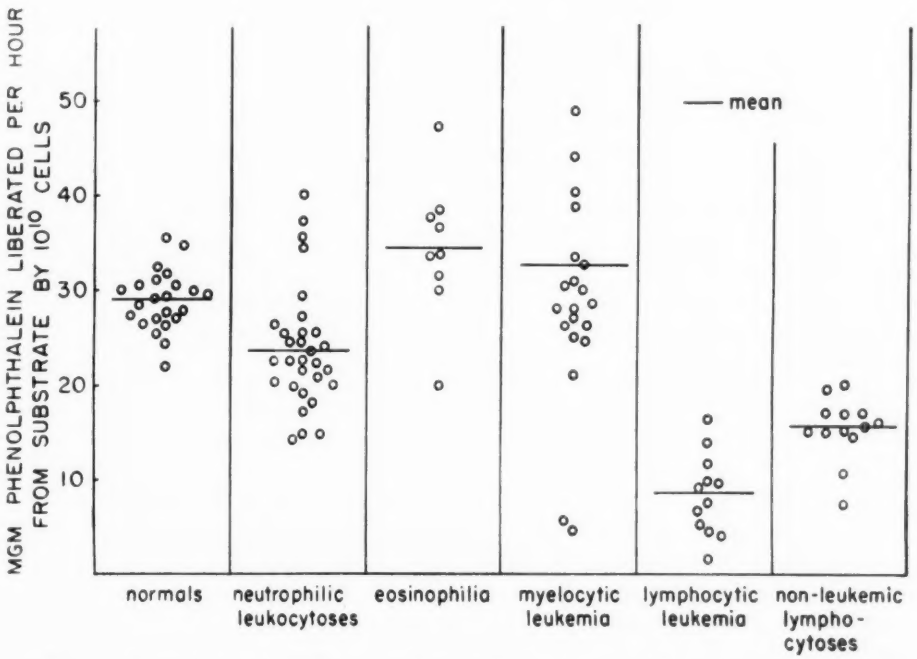

Fig. 1.—Comparison of the β-glucuronidase activity of separated leukocytes from healthy subjects and subjects with hematologic abnormalities associated with various disease states. Values are expressed in terms of milligrams of phenolphthalein liberated per hour from substrate (phenolphthalein-mono-glucuronide) by 10^{10} leukocytes.

β-glucuronidase activity were consistently observed. The highest values were found in chronic myelocytic leukemia and in the eosinophilias. Of interest was the general grouping of patients with neutrophilic leukocytosis at a lower level than normal. Low values were also found in patients with lymphocytosis due to causes other than lymphocytic leukemia.

Normal Subjects.—These 23 subjects had all been admitted to the hospital for nonmedical reasons, i.e., for surgical repair of a hernia, varicose veins, or hemorrhoids. All were in apparent good health at the time the blood sample was drawn. Values for β-glucuronidase activity expressed, as indicated in Methods, varied from 22.0 to 35.6, with a mean value of 28.9. Indicative of the narrow range of activity found to be present in normal subjects is the fact

that 20 of the 23 determinations fell between 24.0 and 32.0. The values per milliliter of blood were found to lie between 11.6 and 32.6 μg per milliliter, with a mean value of 20.7 μg.

Myelocytic Leukemia.—Nineteen determinations were done on 13 patients with chronic myelocytic leukemia. One determination was also done on a patient with subacute myelocytic leukemia. The values found are recorded in Table I. β-glucuronidase activity was found to range from 24.8 to 49.2 in this group. The mean value was 32.6, or 3.7 above the normal mean. However, 13 of the determinations were within the normal range, while only 4 were found to be elevated. Subsequent determinations performed on 2 patients, J. P. and H. H., following marked changes in their clinical and hematologic status, were not included in the above figures, but will be commented on later. The single patient studied with subacute myelocytic leukemia, R. M., was found to have a β-glucuronidase activity of 21.0, a value slightly below normal.

It is apparent that no characteristic abnormal pattern of β-glucuronidase activity is present in patients with chronic myelocytic leukemia, although elevated values may occur. As can be seen in Table I, some patients studied had received no treatment, while others had received variable amounts of therapy with different agents. Some were acutely ill at the time of the determination, while others were reasonably well and able to carry out normal activity. No correlation between enzyme activity and the total leukocyte count could be established. The patients with normal β-glucuronidase activity were found to have leukocyte counts ranging from 9,600 to 309,000 per cubic millimeter, while those with elevated activity showed similar variability, having counts ranging from 7,300 to 343,750 per cubic millimeter. Changes in the relative percentage of cells of the myeloid series other than blast cells had no demonstrable effect on the level of β-glucuronidase. The percentage of mature neutrophilic leukocytes ranged from 12 to 81 in those patients with normal activity, and varied from 14 to 67 in those with elevated activity. Likewise, the relative percentage of stab forms, metamyelocytes, myelocytes or promyelocytes appeared to have no correlation with the β-glucuronidase activity observed.

The most striking observation was the marked fall in β-glucuronidase activity in two patients, J. P. and H. H., who developed acute blastic exacerbations of their disease. J. P. had a normal β-glucuronidase value, 30.6, when first studied. Following the appearance of a high percentage of blasts in his peripheral blood, the β-glucuronidase activity fell to 5.8. H. H. had a high β-glucuronidase value, 40.6, at the time of the initial determination. Three months later, the number of blasts in the differential formula had increased from 4.5 to 86 per cent. The β-glucuronidase fell concomitantly to 4.5. This would appear to indicate a low level of β-glucuronidase in blast forms.

The one patient with subacute myelocytic leukemia, R. M., was found to have 17 per cent blasts in his peripheral blood at the time of the determination. His β-glucuronidase activity was found to be 21.0, slightly lower than normal.

We have been unable to demonstrate any direct correlation between the clinical course of chronic myelocytic leukemia and the level of β-glucuronidase activity, although Anlyan, Gamble, and Hoster[15] have suggested that high

TABLE I. β-GLUCURONIDASE OF LEUKOCYTES IN MYELOCYTIC LEUKEMIA

SUBJECT	W.B.C. PER CU. MM.	DIFFERENTIAL PERCENTAGES										β-GLUCURONIDASE		REMARKS
		BAS.	EOS.	BL.	PRO-MYELOS.	MYELOS.	META-MYELOS.	STABS	SEGS.	LYMPHS.	MONOS.	PER 10^{10} CELLS*	PER ML. BLOOD†	
R. D.	60,250	5.0	2.0	2.0	1.0	31.0	16.0	27.0	12.0	1.0	3.0	25.2	151.4	X-ray therapy
R. D.	8,875	6.0	2.0	2.0	4.0	16.0	14.0	10.0	36.0	6.0	4.0	27.2	24.1	X-ray therapy
R. D.	9,600	9.0	2.0	1.0	2.0	32.0	13.0	10.0	18.0	11.0	2.0	31.0	29.8	X-ray therapy
M. F.	40,000	17.0	3.0	18.0	2.0	22.0	4.0	12.0	14.0	8.0		44.2	168.8	Urethane, P_{32} prev.
M. F.	30,925	13.0	8.0	15.0	3.0	14.0	5.0	12.0	14.0	15.0	1.0	28.3	80.8	X-ray therapy
C. T.	248,333	20.0	4.0	3.0	6.0	17.0	11.0	15.0	21.0	2.0	1.0	26.2	646.0	X-ray therapy
C. T.	88,500	10.0	5.5	2.5	0.5	15.5	3.0	22.0	39.5	1.5		24.8	280.4	X-ray therapy
T. W.	7,300	7.0	2.0			3.0	2.0	8.0	67.0	8.0	3.0	39.4	28.8	X-ray therapy
J. R.	72,500	1.0	1.0	0.5	0.5	22.0	10.0	21.0	43.0	0.5	0.5	28.0	207.6	Urethane
W. A.	12,925	1.5	2.5		0.5	5.0	2.5	4.0	76.0	4.0	4.0	30.2	39.0	Urethane
V. H.	42,500	1.5	0.5		0.5	15.5	14.5	24.0	43.0		0.5	49.2	214.0	Urethane
A. O.	24,500	1.5	1.5			3.0	1.0	1.5	81.5	2.0	8.0	26.4	64.6	Urethane
E. L.	66,000	3.5	0.5		0.5	18.0	10.5	27.5	32.5	2.0	5.0	32.5	214.4	X-ray given 9 mo. prev.
H. H.	243,750	3.0	1.5	4.5	5.0	32.0	9.0	28.0	14.5	2.0	0.5	40.5	1436.0	No therapy
H. H.‡	51,750	0.5	0.5	86.0		1.0	1.5	1.5		7.5	1.5	4.5	23.3	X-ray and cortisone
R. M.	202,000		0.5	17.0	6.0	14.0	12.5	16.0	29.0	4.5	0.5	21.0	424.2	No therapy
J. P.	16,900	4.0	2.0		1.0	12.0	10.0	12.0	50.0	2.0	7.0	30.6	48.5	X-ray therapy
J. P.‡	134,667			70.0		0.5	0.5	4.5	18.0	6.0	0.5	5.8	78.0	X-ray therapy
A. B.	309,000			0.5	1.0	26.5	20.0	28.0	21.5	2.0	0.5	33.6	1038.2	No therapy
L. M.	33,750	1.0	6.0	2.0		7.5	7.0	21.0	48.0	6.5	1.0	28.8	97.2	No therapy

*Expressed as milligrams of phenolphthalein liberated from substrate per hour by 10^{10} cells.
†Expressed as micrograms of phenolphthalein liberated from substrate per hour by the leukocytes of 1 ml. of patient's blood.
‡In acute exacerbation.

β-glucuronidase activity is a poor prognostic sign. In view of the known variability in the course of myeolcytic leukemia, this is difficult to evaluate. Of the four patients found by us to have elevated values, two, V. H. and T. W., have continued to do well, five months in the case of the former, and seven months in the case of the latter, having elapsed since the time of the determination. Patient V. H. had the highest degree of activity in the present series, 49.2, while T. W. was also definitely above the normal range with a value of 39.3. The condition of the third patient, H. H., has progressively deteriorated, but with the acute blastic exacerbation of his disease, β-glucuronidase activity decreased markedly from 40.6 milligrams to a value of 4.5 milligrams. The fourth patient, M. F., proved to have a poor prognosis. He had an initial β-glucuronidase value of 44.2, but this fell to a normal level of 28.4 over a period of one month. Although he received x-ray therapy during this time, his condition continued to deteriorate and he expired shortly thereafter. The initial high value might be said to correlate with the prognosis, but the subsequent fall to normal values prior to his death is not consistent.

Neutrophilic Leukocytosis.—A total of 29 determinations were done on 24 patients with elevations in their total white blood cell counts due to causes other than neoplasm or leukemia. Leukocyte counts ranged from 10,825 to 34,850 per cubic millimeter, while β-glucuronidase values for the whole group varied from 14.4 to 40.4. The mean value was 23.8, or approximately 5.0 lower than the mean for the normal patients studied.

In general, the cases fell into two groups: (1) those with miscellaneous causes of leukocytosis, and (2) those with leukocytosis secondary to infection. The former group included patients with cirrhosis of the liver, bleeding esophageal varices, acute cerebrovascular accident, and fractures. In 8 patients studied, the range of values was found to be 19.2 to 37.4, with a mean of 24.7. Two patients, F. J. and V. E., had levels of activity below the lowest normal, 21.6 and 19.2, respectively, while the others were grouped for the most part in the low normal range.

The latter group consisted of patients with an acute infectious process such as pneumonitis, meningitis, pyelonephritis, tonsillitis, septicemia, or pyogenic complications of surgery. Twenty-two determinations were done on the 16 patients in the group. Although a relatively wide range of activity was encountered, 8 of the patients had values below those in any normal subject on one or more occasions during their illness. The range was 14.4 mg. to 40.4, while the mean value was 23.3. All values in both groups are recorded in Table II.

There is no definite correlation between either the total leukocyte count or the differential formula. Low values were encountered with counts as low as 13,550 per cubic millimeter (patient F. DeF.) and as high as 27,193 per cubic millimeter (patient E. P.). The poor correlation between the total leukocyte count and the β-glucuronidase level is illustrated by the fact that of the 12 determinations done on patients with leukocyte counts above 18,000 per cubic millimeter 7 were found to be low, while 5 were within the normal range.

TABLE II. β-GLUCURONIDASE OF LEUKOCYTES IN DISEASE STATES WITH NEUTROPHILIC LEUKOCYTOSIS

SUBJECT	W.B.C. PER CU. MM.	DIFFERENTIAL PERCENTAGES										β-GLUCURONIDASE		DIAGNOSIS
		BAS.	EOS.	BL.	PRO-MYELOS.	MYELOS.	META-MYELOS.	STABS	SEGS.	LYMPHS.	MONOS.	PER 10^{10} CELLS*	PER ML. BLOOD†	
A. S.	19,250						4.0	13.0	70.0	6.0	7.0	22.8	45.5	Cerebral hemorrhage
G. M.	10,825		3.0			1.0	3.0	25.0	49.0	18.0	1.0	37.4	40.5	Bleeding esoph. varices
F. J.	15,525	2.0	1.0			1.0	1.0	9.0	68.0	13.0	5.0	21.6	33.4	P.O. cholecystectomy
C. B.	15,525	0.5	3.0					15.5	69.0	9.0	3.0	24.6	36.8	Hepatic decompensation
E. C.	19,225						2.0	13.5	68.5	4.0	12.0	25.6	49.2	Hepatic decompensation
T. B.	16,850							11.5	80.0	0.5	8.0	22.3	37.5	P.O. gastric resect.
V. E.	20,100						0.5	9.0	77.5	11.0	2.0	19.2	38.4	Cirrhosis of the liver
E. H.	11,225					0.5	0.5	6.0	76.0	10.5	6.5	24.2	26.5	Fracture
F. B.	17,500						1.0	16.0	67.0	5.0	11.0	34.2	59.8	Tonsillitis, acute
F. D.	13,550	2.0	1.0					14.0	57.0	13.0	13.0	18.0	24.4	Cholangitis
L. A.	22,575	1.0	1.0				1.0	14.0	72.0	5.0	6.0	14.6	32.9	Pelvic abscess
L. A.	29,375		1.0				1.0	28.0	49.0	11.0	10.0	25.8	75.8	Pelvic abscess
L. A.	34,850		1.0					36.0	47.0	8.0	8.0	22.3	77.6	Pelvic abscess
R. G.	23,750	1.0	3.0				1.0	21.0	21.0	38.0	15.0	23.2	55.0	Ulcerative colitis
E. P.	27,193						3.0	8.0	75.5	8.0	5.5	20.9	56.4	Acute pneumonitis
L. W.	22,525					0.5	1.5	20.0	55.5	12.0	10.5	24.1	53.2	Tonsillitis
E. S.	27,750					1.0	3.5	20.0	69.0	2.5	4.0	27.4	75.8	Meningitis
E. S.	18,000		0.5					11.0	80.0	4.5	4.0	17.2	31.2	Meningitis
E. S.	7,387	0.5	9.0					6.5	67.5	12.5	4.0	25.6	18.9	Meningitis, convalescing
F. R.	12,925		0.5					5.0	68.0	21.5	5.0	40.4	52.6	Urinary tract infection
H.D.	12,987							1.0	83.0	14.0	2.0	35.6	46.3	Upper resp. infection
T. C.	14,175	1.0	0.5					0.5	86.5	8.5	3.0	19.2	28.3	P.O. cholecystectomy
T. C.	22,000						0.5	7.0	85.5	2.0	5.0	14.8	32.4	P.O. cholecystectomy
T. C.	18,675							6.0	78.5	15.0	0.5	14.4	26.8	P.O. cholecystectomy
C. M.	17,750		0.5			0.5		21.0	64.0	7.5	6.5	22.4	39.8	Pneumonitis
W.B.	16,000		0.5				0.5	6.5	85.5	3.5	3.5	20.1	32.4	Pyelonephritis
J.M.	12,125	1.0	0.5					8.0	69.0	13.5	8.0	29.2	35.4	Acute appendicitis
J. K.	19,425							13.0	78.0	4.0	5.0	20.3	39.5	Ulcerative colitis
T. P.	16,500	0.5	3.5					12.0	65.5	16.0	2.5	26.3	43.3	Coccidioides
P. B.	22,550		0.5				1.5	25.0	54.0	13.0	6.0	21.3	47.8	Pyelonephritis

Range of β-glucuronidase activity per 10^{10} cells: 14.4 to 40.4
Mean β-glucuronidase value per 10^{10} cells: 23.8

*Expressed as milligrams of phenolphthalein liberated from substrate per hour by 10^{10} cells.
†Expressed as micrograms of phenolphthalein liberated from substrate per hour by the leukocytes of 1 ml. of patient's blood.

In spite of this poor correlation with the total or differential count, the low values in 2 patients of the group with nonspecific leukocytosis, and in approximately 50 per cent of those with leukocytosis of infection did show some correlation with the patient's clinical status and the severity of the disease process. In general, severe infections and complications were associated with diminished β-glucuronidase activity of the leukocytes, and this returned in the direction of normal with improvement in the clinical status. This is illustrated by the findings in the following 3 patients:

One of the patients studied, T. C., was found to have persistently low β-glucuronidase values on three occasions. These were obtained over a three-week period following a cholecystectomy which was complicated by the occurrence of a severe urinary tract infection and septicemia. Although antibiotics were administered, the clinical course was critical at all times. At the time of the first determination on April 25, 1952, the leukocyte count was 14,175 per cubic millimeter, with a β-glucuronidase activity of 19.2. On May 6, 1952, his count had become elevated to 22,000 per cubic millimeter, and β-glucuronidase activity had fallen to 14.8. A third determination done ten days later was found to be at the low level of 14.4. The total leukocyte count was 18,675 per cubic millimeter.

The second patient, E. S., demonstrated not only a fall in β-glucuronidase activity in response to acute infection, but also showed a return to normal with recovery. He was admitted to the hospital on March 16, 1952, with a diagnosis of acute meningitis of recent onset. On March 17, 1952, his leukocyte count was 27,750 per cubic millimeter and his β-glucuronidase activity was 27.4. During the next few days his hospital course was stormy, being complicated by the necessity for a tracheotomy to alleviate tracheobronchial obstruction. His leukocyte count on March 24, 1952, was still elevated to 18,000 per cubic millimeter, and β-glucuronidase activity had decreased to 17.2. By the nineteenth hospital day the patient's clinical condition had improved markedly, so that he was fully conscious and able to feed himself. The leukocyte count had fallen to 7,387 per cubic millimeter, and the β-glucuronidase had increased to a normal value of 25.6.

The third patient, L. A., demonstrated a definitely low value of β-glucuronidase activity on only one occasion during the period of study, although leukocytosis was present at the time of each determination. This 30-year-old white man had an appendectomy on Sept. 5, 1951, followed by the development of a large pelvic abscess as a postoperative complication. This was evacuated surgically on Sept. 15, 1951. On September 21 his leukocyte count was 22,575 per cubic millimeter and β-glucuronidase activity at that time only 14.58. By September 23 his general condition had improved considerably so that a Harris tube could be removed. Two days later his leukocyte count had increased to 29,375 per cubic millimeter, but the β-glucuronidase activity was found to be normal (25.8). By September 27 he had become much worse clinically, and it was necessary to reinsert the Harris tube. His temperature reached 101.8° F., and a fecal odor was noted to be coming from his wound. The total white blood count was 34,850 per cubic millimeter and β-glucuronidase activity had de-

creased to 22.3. Another small intra-abdominal collection of pus was found at laparotomy the following day, and the patient then went on to complete recovery.

Eosinophilia.—The effect of an increase in eosinophil percentage on the level of β-glucuronidase activity was investigated. The results found in the 7 cases studied are indicated in Table III.

A total of nine determinations were done and the values were found to range from 19.9 to 47.2 The mean for the group was 34.2. This was 5.3 higher than the mean value for the normal patients studied. Four of the patients had values well above the normal range on at least one occasion, while all values except one were above 30.8, a relatively high normal value.

The cause of the eosinophilia in the patients investigated was variable. Included in the study were cases with eosinophilia due to trichinosis, bronchial asthma, osteomyelitis, and erythema multiforme. In three patients the etiology was undetermined, however, in spite of extensive clinical and laboratory studies. In one of these, patient B. D., peripheral blood and bone marrow smears were inconclusive, and he may eventually prove to have an atypical myelocytic or eosinophilic leukemia. He was included in the group, however, because of the high percentage of circulating eosinophils (46 per cent). The diversity in etiology of the cases studied permits no conclusion as to the influence of the specific disease entities on the β-glucuronidase content of the leukocytes. The data would appear to indicate, however, that the eosinophil has at least a normal or slightly high content of β-glucuronidase.

Chronic Lymphocytic Leukemia.—A total of 12 determinations were done on 11 patients with chronic lymphocytic leukemia. The values for β-glucuronidase activity were uniformly low, as illustrated by Table IV. Values ranged from 1.5 to 16.1. The mean value was 8.3 or less than one-third the normal mean value. Patient A. C., who had 99 per cent circulating small mature lymphocytes, was observed to have the lowest value. It seems clear that the lymphocytes of chronic lymphocytic leukemia are low in β-glucuronidase activity. Whether this is a reflection of cell type or of the disease itself cannot be determined with certainty.

Acute Leukemia.—Patient M. P., with acute lymphocytic leukemia, was studied with repeated determinations. The level of β-glucuronidase activity was found to be very low in the presence of a high percentage of blast forms, 2.8 milligrams. Following treatment with A-methopterin, his leukocyte count fell from 277,550 to 17,775 per cubic millimeter, and the percentage of blasts was reduced from 91 per cent to 37 per cent. β-glucuronidase activity rose concomitantly to 8.4. With further improvement, β-glucuronidase activity increased to 15.2, but was again reduced to 9.5, following hematologic relapse. Normal values were not observed, although Anlyan, Gamble, and Hoster[15] found normal levels of activity in all cases of acute lymphocytic leukemia studied by them.

Patient A. V., with leukemia of undetermined cell lineage, ran a very acute course while in the hospital. At the time of the determination, 96 per cent blast forms were present on the peripheral smear. β-glucuronidase activity was low,

TABLE III. β-GLUCURONIDASE OF LEUKOCYTES IN DISEASE STATES WITH EOSINOPHILIA

SUBJECT	W.B.C. PER CU. MM.	DIFFERENTIAL PERCENTAGES										β-GLUCURONIDASE		DIAGNOSIS
		BAS.	EOS.	BL.	PRO-MYELOS.	MYE-LOS.	META-MYELOS.	STABS	SEGS.	LYMPHS.	MONOS.	PER 10^{10} CELLS*	PER ML. BLOOD†	
A. H.	9,975		33.0					2.0	36.0	20.0	9.0	38.6	38.2	Etiology unknown
A. H.	6,900		28.0				3.0	2.0	29.0	33.0	5.0	30.8	21.3	Etiology unknown
D. B.	10,975	1.0	30.0			2.0	1.0	6.0	34.0	23.0	3.0	36.8	40.4	Trichinosis
D. B.	11,000		33.0			1.0	3.0	2.0	29.0	29.0	3.0	33.9	37.2	Trichinosis
C. L.	13,400	1.0	17.0			1.0	1.0	5.0	66.0	8.0	1.0	19.9	26.0	Erythema multiforme
E. M.	7,525	1.0	10.0					7.0	42.0	33.0	7.0	31.5	23.5	Bronchial asthma
A. W.	10,950	2.0	16.0					4.0	37.0	24.0	17.0	37.8	50.4	Diabetes and osteomyelitis
N. B.	9,800	0.5	23.5					4.0	41.0	24.0	7.0	33.5	32.8	Etiology unknown
B. D.	60,625		46.0				0.5	8.5	27.0	4.0	14.0	47.2	286.0	Etiology unknown

Range of β-glucuronidase activity per 10^{10} cells: 19.9 to 47.2
Mean β-glucuronidase value per 10^{10} cells: 34.2

*Expressed as milligrams of phenolphthalein liberated from substrate per hour by 10^{10} cells.
†Expressed as micrograms of phenolphthalein liberated from substrate per hour by the leukocytes of 1 ml. of patient's blood.

TABLE IV. β-GLUCURONIDASE OF LEUKOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA

SUBJECT	W.B.C. PER CU. MM.	DIFFERENTIAL PERCENTAGES										β-GLUCURONIDASE		REMARKS
		BAS.	EOS.	BL.	PRO-MYELOS.	MYELOS.	META-MYELOS.	STABS	SEGS.	LYMPHS.	MONOS.	PER 10^{10} CELLS*	PER ML. BLOOD†	
G. .M	7,800			2.0					2.0	96.0		7.6	17.6	
M. F.	12,600		2.0					1.0	22.0	70.0	5.0	9.8	12.4	
M. K.	345,750			3.0				1.0	1.0	95.0		4.5	156.3	
M. K.	298,750		1.0	1.0				1.0	2.0	95.0		4.2	124.8	
H. K.	33,000	1.0	2.0				1.0	1.0	9.0	84.0	2.0	11.5	37.8	
A. T.	59,475							2.0	12.0	83.0	3.0	6.8	40.4	
A. C.	29,875									99.5	0.5	1.5	7.7	
M. S.	33,000							4.0	13.0	80.0	3.0	9.0	29.6	
L. H.	12,350								10.0	88.5	1.5	14.3	17.6	
L. I.	23,550	0.5	4.0					0.5	34.5	59.5	1.0	16.1	38.0	
L. K.	67,950		1.0					0.5	5.5	92.0	1.0	5.1	34.8	
B. B.	73,000							1.0	9.5	88.5	1.0	9.3	67.6	
		Range of β-glucuronidase activity per 10^{10} cells: 1.5 to 16.1												
		Mean β-glucuronidase value per 10^{10} cells: 8.3												
		β-Glucuronidase of leukocytes in acute lymphocytic leukemia												
M. P.	277,550			91.0	1.0	1.0		2.0		5.0		2.8	79.4	
M. P.	17,775		0.5	37.0			0.5	1.0	8.0	52.0	1.0	8.4	14.8	A-methopterin
M. P.	30,775	3.0		9.0			1.0	3.0	42.0	41.0	1.0	15.2	43.0	A-methopterin
M. P.	66,500		2.0	5.0				2.0	26.0	65.0		9.5	63.4	A-methopterin
		β-Glucuronidase of leukocytes in blast cell leukemia of undetermined type												
A. V.	221,750			96.0				1.5	0.5	2.0		4.2	92.8	

*Expressed as milligrams of phenolphthalein liberated from substrate per hour by 10^{10} cells.
†Expressed as micrograms of phenolphthalein liberated from substrate per hour by the leukocytes of 1 ml. of patient's blood.

TABLE V. β-GLUCURONIDASE OF LEUKOCYTES IN INFECTIOUS MONONUCLEOSIS

SUBJECT	W.B.C. PER CU. MM.	DIFFERENTIAL PERCENTAGES										β-GLUCURONIDASE		REMARKS
		BAS.	EOS.	PRO-MYELOS.	MYE-LOS.	META-MYELOS.	STABS	SEGS.	LYMPHS.	ATYP-ICAL LYMPHS.	MONOS.	PER 10^{10} CELLS*	PER ML. BLOOD†	
J. M.	12675					1.0	6.0	8.0	19.0	61.0	5.0	18.2	23.1	
J. M.	8825	1.0	1.0			1.0	7.0	20.0	45.0	21.0	4.0	15.0	13.2	
J. H.	11650	1.0	3.0			1.0	9.0	21.0	13.0	47.0	5.0	18.0	21.0	
A. C.	8375	1.0	0.5				16.5	33.5	27.5	16.0	5.0	18.3	15.3	
A. A.	9675	1.0	0.5				3.5	24.5	10.5	58.0	2.0	15.2	14.7	
E. M.	7825		0.5				10.0	2.0	47.0	32.0	8.5	7.6	5.9	
Range of β-glucuronidase activity per 10^{10} cells: 7.6 to 18.3														
Mean β-glucuronidase value per 10^{10} cells: 15.4														
β-Glucuronidase in disease processes with nonspecific lymphocytosis														
M. P.	3350		3.0			1.0	14.0	27.0	52.0		3.0	14.5	4.9	Hypoplastic anemia
M. P.	3675	1.0	2.0			1.0	10.0	28.0	46.0		12.0	20.2	7.4	Hyoplastic anemia
A. I.	16750	1.5					2.5	25.0	67.5		3.5	15.2	25.5	Infection ? etiology
D. S.	17250	0.5	1.0		1.5	1.0	1.0	18.0	71.0		6.0	14.8	25.5	Lymphocytosis secondary to chronic dermatitis
R. W.	12650						18.5	35.5	32.5	9.5	4.0	19.6	23.5	Guillain-Barré Syndrome (50 per cent lymphs. prev. day)
S. G.	1050						7.0	5.0	88.0			10.4	1.9	Aplastic anemia
P. S.	1712	1.0	1.0			0.5	6.0	28.0	58.0		5.0	15.8	2.7	Aplastic anemia
Range of β-glucuronidase activity per 10^{10} cells: 10.4 to 20.2														
Mean β-glucuronidase value per 10^{10} cells: 15.8														

*Expressed as milligrams of phenolphthalein liberated from substrate per hour by 10^{10} cells.
†Expressed as micrograms of phenolphthalein liberated from substrate per hour by the leukocytes of 1 ml. of patient's blood.

being 4.2. The occurrence of low values in association with high blast percentages as observed in these two patients indicates that not only the myeloblast (as observed in acute exacerbations of chronic myelocytic leukemia) but also blast forms in general probably have a low content of β-glucuronidase.

Lymphocytosis.—A total of 13 determinations were done on 11 patients with relative or absolute lymphocytosis due to causes other than lymphocytic leukemia (Table V). Five of these had infectious mononucleosis. Five of the remaining patients with lymphocytosis had peripheral lymphocyte percentages of over 50 per cent. In the eleventh subject, R. W., lymphocytes contributed 42 per cent of the leukocytes at the time of the determination, although over 50 per cent had been present the previous day. This patient had ascending paralysis of the Guillain-Barré type, probably due to infectious mononucleosis, but the diagnosis was never unequivocally established. In the other cases, the lymphocytosis was found associated with infection, chronic dermatitis, and hypoplastic or aplastic anemia.

The values in the patients with infectious mononucleosis were found to range from 7.6 to 18.3 with a mean of 15.4 (Table V). The values in the group with miscellaneous causes of lymphocytosis ranged from 10.4 to 20.2 with a mean value of 15.8. All values observed were definitely below normal. The mean value for the patients with infectious mononucleosis was approximately 12.0 below the normal mean, although it was approximately twice the mean value for the group with chronic lymphocytic leukemia. The low β-glucuronidase values found in the presence of lymphocytosis of diverse origin would seem to indicate that such lowered activity is either due to a nonspecific effect in conditions in which a lymphocytic response is called forth, or else is due to a low content of β-glucuronidase in the lymphocyte per se.

Miscellaneous Conditions and Collagen Diseases.—No striking deviations from normal glucuronidase values were observed in patients with collagen disease, irrespective of whether or not the patient was receiving cortisone. Essentially normal values were also found in subjects with Hodgkin's disease, diabetes mellitus, polycythemia, carcinoma, and reticulum-cell sarcoma. One patient with lymphosarcoma, and another patient with multiple myeloma were both observed to have low values. The findings in another patient with multiple myeloma were in the normal range, however.

DISCUSSION

β-glucuronidase activity of considerable magnitude is present in the separated leukocytes of normal human subjects with a variety of hematologic disorders and miscellaneous disease states. Activity in disease exhibits variations from normal which may be due in part to the underlying disease process, and in part to differences in the proportion of various leukocyte types present in the samples analyzed. When the values obtained in normal subjects in these studies are compared with those of Rossiter and Wong,[12] it is noted that they are of considerably greater magnitude. In both studies nearly the same type of incubation mixture was employed, and in both activity was expressed in terms of cell numbers. It is not clear why such differences exist, although different

techniques were employed in isolating and preparing leukocyte suspensions. Care was taken in these studies to wash the leukocytes only once before resuspending, since Fishman and associates[11] have shown that repeated washing decreases measurable enzyme activity, presumably through the removal of some necessary dialysable cofactor. Using suspensions of leukocytes ranging from 5,000 to 15,0000 per cubic millimeter has uniformly permitted satisfactory colorimetric readings with an incubation period of three hours. Since the investigations of Rossiter and Wong dealt only with the leukocytes of normal subjects, further comparison of data obtained in various disease states is not possible.

Comparison of the data obtained in these studies with those of Anlyan and co-workers[15] is difficult in absolute terms since there are differences in technique involved, and since the standards of reference are not the same. The data of Anlyan and collaborators are expressed in terms of wet weight of tissue, and in our studies in terms of activity per 10^{10} separated leukocytes. On a comparative basis, there is agreement on some points and disagreement on others. The data obtained serve to emphasize the following points of interest:

1. Although Fishman and co-workers[5] have observed a general elevation in β-glucuronidase activity in neoplastic tissue, marked contrast exists between the values obtained in chronic lymphocytic leukemia, blastic leukemia, and chronic myelocytic leukemia. In the former two situations, very low levels of activity were observed, while in the latter normal or elevated values were noted. The finding of low values in chronic lymphocytic leukemia is in accord with the observations of Anlyan and co-workers,[15] but the normal to high values, observed in chronic myelocytic leukemia, is at variance with the low values obtained by these workers. Further, in our cases of acute leukemia and acute exacerbations of chronic myelocytic leukemia, low values were found in contrast to the normal values reported in Anlyan's series.

The data on chronic lymphocytic leukemia would seem to indicate either (a) the circulating lymphocyte per se is fundamentally poor in β-glucuronidase activity, or (b) the pathologic lymphocyte of leukemia is metabolically different from the normal lymphocyte, having a lower glucuronidase activity. The statistical treatment of data on normal subjects by the technique of regression equations, reported by Rossiter and Wong,[12] is interpreted to mean that normal lymphocytes contain nearly as much demonstrable β-glucuronidase activity as do the neutrophiles of the circulating blood. This would support the second postulate. However, in the studies reported here, uniformly low values for β-glucuronidase activity have been noted wherever lymphocytosis was present, irrespective of the etiology of the lymphocytosis, whether due to leukemia, infectious mononucleosis, or other lymphocytosis of nonleukemic origin. It may be argued that in all these situations the biochemical constitution of the lymphocytes may be different from normal, but some reservations must be retained as to the ultimate answer to the problem until satisfactory methods of separating the various leukocyte types of normal blood are available. In this regard, it should also be noted that a heavy concentration of β-glucuronidase activity has

been demonstrated in the Malpighian corpuscles of the spleen where the lymphocyte is the predominant cell type.[21] On the other hand, Fishman and Anlyan[4, 5] have found the β-glucuronidase activity of lymph node tissue to be low. This, together with findings on leukocyte suspensions from peripheral blood in situations where the lymphocyte is the predominant cell type, raises the question of the metabolic and functional interrelationships of splenic, nodal, and circulating lymphocytes.

2. Comparison of the β-glucuronidase activity of leukocyte suspensions composed of widely differing leukocyte types with variable stages of maturation and development leads to certain inferences as to β-glucuronidase activity of these variable cell types at different stages of development. The presence of normal or slightly elevated values in chronic myelocytic leukemia where large numbers of relatively immature cells of the myeloid series above the blast state are present suggests that a normal complement of β-glucuronidase is present early in the development of this cell type. The low activities observed in acute blastic leukemias and in acute blastic exacerbations of chronic myelocytic leukemia suggest that the blast cell, unlike its more differentiated progeny, may be relatively poor in β-glucuronidase activity. The finding of normal and elevated values in leukocyte suspensions containing a high percentage of eosinophils is most easily interpreted as meaning that the eosinophil, as well as the neutrophilic polymorphonuclear leukocyte, contains a substantial amount of this enzyme. The inferences to be derived from the findings on suspensions composed largely of lymphocytes have been previously discussed. It must be admitted, however, that in all these circumstances, the relative importance of underlying pathology and dominant cell type cannot be effectively separated with certainty.

3. In these studies it has not been possible to confirm the relationship of β-glucuronidase activity to prognosis in chronic myelocytic leukemia, as reported by Anlyan and co-workers.[15] The observation by these workers of high values in the preterminal stage of the disease was not apparent in those cases in this series in which this could be evaluated. In fact, blastic exacerbation and its associated poor prognostic import appeared to be accompanied by a marked diminution in unit cell β-glucuronidase activity. During infectious processes of a serious and critical nature, low values were observed, and these returned in the direction of normal with clinical improvement.

4. In patients with miscellaneous disease states receiving cortisone in large doses, the β-glucuronidase activity was not outside the normal range.

The possible functional implications of β-glucuronidase activity in white blood cells has been discussed by Rossiter and Wong.[12] It is well known, of course, that glucuronide conjugation is an important mechanism of detoxification. However, while Fishman has championed the possible synthetic functions[12, 21] of β-glucuronidase, much of the evidence to date emphasizes the hydrolytic[23, 24] rather than synthetic capacities of the enzyme. The metabolic significance of glucuronide hydrolysis is not so clear. The variations in β-glucuronidase activity of leukocytes in health and disease must, therefore, for

the present, be presented on a descriptive basis with the hope that further knowledge on the role of glucuronidase will eventually permit a functional interpretation.

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CHANGES IN WHITE BLOOD CELL COUNTS AFTER ADMINISTRATION OF CORTISONE ACETATE TO HEALTHY AMBULATORY INDIVIDUALS

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INTRODUCTION

ALTERATIONS in adrenal cortical function are accompanied by fluctuations in white blood cell counts of peripheral blood. In hypercorticism, e.g., Cushing's syndrome, granulocytosis occurs with lymphopenia and eosinopenia.[1] In hypoadrenal states, such as Addison's disease or panhypopituitarism, low total white blood cell counts with neutropenia and lymphocytosis are typical findings.[1] An increased eosinophil count has been observed frequently in Simmonds' disease.[2]

The administration of adrenocorticotrophic hormone to man and animals regularly produces an increase in neutrophils and decrease in lymphocytes and eosinophils.[3-10]

The intramuscular administration of cortisone acetate is also followed by changes in white blood cell counts. Perera and associates[11] observed neutrophilia and eosinopenia, but no marked changes in lymphocyte counts, in one patient with Addison's disease and in two hypertensive patients during treatment with cortisone acetate intramuscularly (20 mg. every six hours for eight days). Luetscher and Deming[12] noted eosinopenia and increased total white blood cell counts in 8 of 9 nephrotic patients who received cortisone acetate intramuscularly (100 mg. daily for five or six days). Sprague and co-workers[13] reported consistent increases in the total white blood cell counts of 33 patients during prolonged treatment with cortisone. Neither lymphopenia nor eosinopenia was a constant finding in their experience. However, many other observers[14-17] have noted eosinopenia during therapy with cortisone acetate. Four patients with Addison's disease were treated by Thorn and Forsham[18] with intramuscular injections of cortisone acetate (100 mg. daily for five days). In three of the four patients, they observed falls in the eosinophil counts representing from 20 to 50 per cent of the pretreatment values. Although the times of these observations were not indicated in the published report, the results were stated to be in marked contrast to the striking declines noted at four hours after the intravenous administration of cortisone acetate. Thorn and collaborators[19] have also reported that the eosinophil counts of patients with Addison's disease show greater declines at four hours after the oral administration of cortisone acetate than after

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intramuscular injection of the drug in the same doses. In a more recent publication[20] they concluded that the eosinopenic effect of oral administration of cortisone acetate approximates the effect of intravenous administration both in magnitude and time of response.

From all of these observations of the administration of cortisone to patients with a variety of diseases, it appears that: (a) Intramuscular injection of cortisone acetate usually has been followed by an increase in the neutrophil count and, inconstantly, by a decrease in the lymphocyte and eosinophil counts. (b) Cortisone acetate probably exerts its effect on white blood cell counts less promptly after intramuscular than after oral or intravenous administration.

PRELIMINARY OBSERVATIONS

Incidental casual observations in this laboratory during another study suggested that normal ambulatory medical students have a more marked rise in neutrophil count after intramuscular than after oral administration of cortisone acetate. These preliminary observations were made on subjects receiving cortisone acetate in divided doses, totaling 200 mg. daily, for about five days. The aqueous vehicle, in which the standard preparation of cortisone acetate is suspended for intramuscular use, was given intramuscularly to a few of the subjects in doses of 2 ml. four times daily.* Fig. 1 shows the median counts of total white blood cells, neutrophils, and lymphocytes for the combined data on six subjects plotted against time in days. The highest neutrophil counts occurred after cortisone acetate was given intramuscularly, although oral administration of the drug was also accompanied by a rise in neutrophil counts. There were no consistent changes in these cell counts after injection of the aqueous vehicle.

All of those subjects who received cortisone acetate intramuscularly complained of discomfort at the sites of injection in the upper outer quadrants of the buttocks. This distress began several hours after the first injection, gradually increased throughout the day, and persisted for about two days after termination of the treatment. The subjects tended to walk with a wide base and complained particularly about the distress when having to climb stairs. They could obtain temporary relief by applying heat to the painful sites. There was no definite evidence of suppuration at any time. None of the subjects had any complaints after the oral administration of cortisone acetate or after intramuscular injection of the aqueous vehicle.

The present controlled study was undertaken to extend the description of changes in white blood cell counts on peripheral blood of healthy ambulatory men after treatment with cortisone acetate, to compare the effects of route of administration on white blood cell counts and to verify the pronounced increase in the neutrophil count which had been observed to follow the intramuscular injection of cortisone acetate.

*Both oral and intramuscular cortisone acetate and the aqueous vehicle were generously supplied by Merck and Company, Inc., Rahway, N. J., through the courtesy of Dr. Augustus Gibson. The vehicle consisted of sodium carboxymethyl cellulose, Tween 80, and 1.5 per cent benzyl alcohol in isotonic saline.

MATERIALS AND METHODS

Single doses of oral cortisone acetate (50 mg.), intramuscular cortisone acetate (50 mg. in 2 ml.), and aqueous vehicle (2 ml.) were each given on successive occasions to eight healthy ambulatory medical students. The six possible orders in which the three test materials could be given were arranged systematically in eight sets, and these were assigned at random to the eight subjects (Table I). The assignment of the test materials was unknown to the laboratory technicians performing the cell counts. There was an interval of at least forty-eight hours between the successive periods of observation.

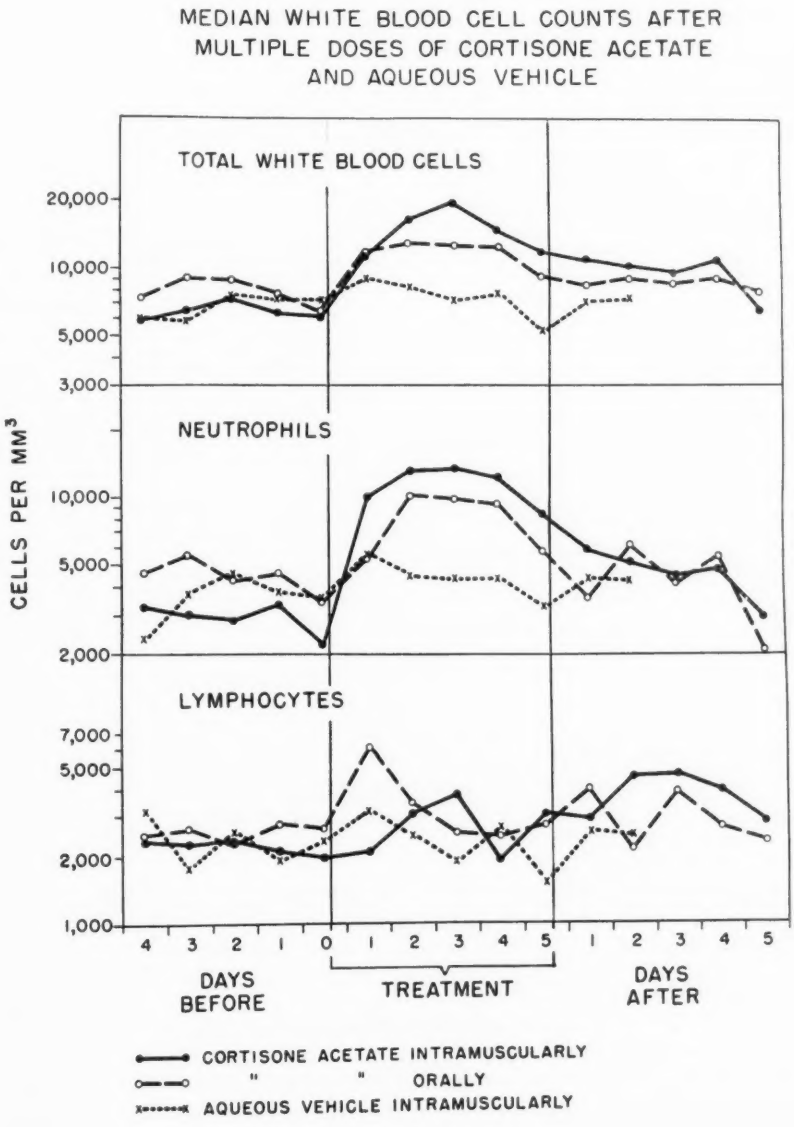

Fig. 1.

A sample of capillary blood was taken by a technician between 8:00 and 8:30 A.M., just prior to administration of the test material, and further samples were obtained at intervals of one or one and one-half hours during the day until about 4:30 P.M. These samples were used for total white blood cell, eosinophil, and differential counts. During the time of day when the technicians were not present, 5 ml. of venous blood were collected by medical students at approximately 7:00 P.M., 10:00 P.M., and midnight. Blood films were made immediately from a few drops, and the remainder was preserved in the double oxalate mixture of Heller and Paul[21] for counting total white blood cells and eosinophils the next morning. A final sample of capillary blood was taken by a technician about twenty-four hours after the test material had been given.

TABLE I. ORDER OF ADMINISTRATION OF TEST MATERIALS TO HEALTHY AMBULATORY SUBJECTS

SUBJECT	TEST MATERIAL NO. 1	TIME INTERVAL (DAYS)	TEST MATERIAL NO. 2	TIME INTERVAL (DAYS)	TEST MATERIAL NO. 3
1	V	2	O	2	I
2	O	2	I	2	V
3	V	2	O	2	I
4	V	2	I	2	O
5	I	6	V	2	O
6	V	2	I	2	O
7	I	2	O	2	V
8	O	2	V	2	I

V = 2 ml. of the aqueous vehicle, intramuscularly.
O = 50 mg. of cortisone acetate, orally.
I = 50 mg. of cortisone acetate, intramuscularly.

Two Bard-Parker pipettes were used for each total white blood cell count. Both chambers of a calibrated Neubauer hemocytometer, 0.1 mm. in depth, were filled from each pipette. The mean of the four chamber counts was recorded as the total white blood cell count. The diluting solution of 2 per cent acetic acid was freshly prepared each morning from glacial acetic acid and distilled water.

For the eosinophil count, two Bard-Parker white blood cell pipettes were filled with blood to the "1.0" mark and diluted with Speirs and Meyer's solution[22] as modified by Kass, Lundgren, and Finland.[23] A calibrated Neubauer hemocytometer was charged on both sides from one pipette. The number of eosinophils counted in the eight peripheral squares of both chambers (1.6 c.mm.) was multiplied by 6.25 to give the number of cells per cubic millimeter of blood. The mean of two such hemocytometer counts was recorded as the eosinophil count.

Cover slip films of blood were prepared with Wright's stain for differential counting. The percentages, used to compute the recorded estimates of the absolute neutrophil and lymphocyte counts, were based upon the counting of 400 white blood cells.

RESULTS

I. *Objective Findings.*—

The data collected in this study are summarized in Table II and are presented graphically in Fig. 2. The test materials are shown in the first column of Table II. The period of observation after treatment has been divided into time zones numbered "1" through "7," as shown in column "2." The first four zones, each of two hours duration, extend from a half hour to eight and one-half hours after administration of the test material at zero time. Zones "5" and "6" are each four hours long, the latter zone ending at sixteen and one-half hours after zero time. No further observations were made until the final counts in zone "7," which extends from twenty-three and one-half to twenty-six and one-half hours after zero time. Zero time and the midpoints of the time zones in "hours

after zero time" are indicated in column "3." An arithmetic mean was computed for each cell type from the cell counts* of all of the subjects who were observed in a particular time zone after the same treatment. These mean counts are tabulated† in columns "4" through "7" of Table II as neutrophils, lymphocytes,

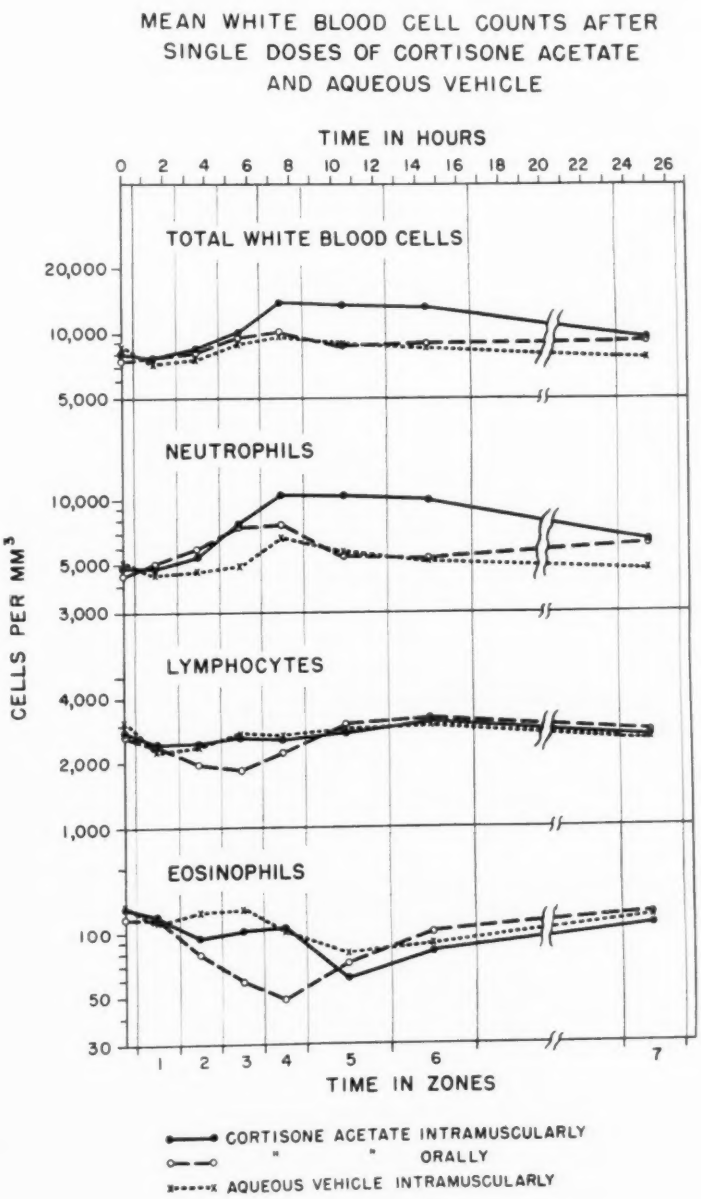

Fig. 2.

*In any time zone in which more than one count of a particular cell type had been made in any subject, the average of the several values was recorded as the cell count for that subject.

†The original data, from which the tabulated values have been computed, will be sent to the interested reader upon request.

eosinophils, and total white blood cells, respectively. In Fig. 2, the mean counts are plotted at the midpoints of the time zones, according to cell type and test material.

A. *Intramuscular Administration of Aqueous Vehicle:* The white blood cell counts, observed after the intramuscular administration of the aqueous vehicle, were used as a base line for evaluating the changes in cell counts after cortisone acetate was given orally and intramuscularly. These base line trends were probably determined in part by diurnal variations of the cell counts.

The neutrophil count remained near the initial level of 5,070 cells per cubic millimeter for about five and one-half hours after the intramuscular administration of the aqueous vehicle. An increase to 6,740 cells per cubic millimeter was observed at about seven and one-half hours after zero time (zone 4), after which the count returned gradually toward the initial level.

Fluctuations in the lymphocyte count throughout the day did not show any striking deviations from the initial value of 3,100 cells per cubic millimeter, although the data suggest a slight decrease during the first four hours after injection of the vehicle.

TABLE II. MEAN WHITE BLOOD CELL COUNTS, IN CELLS PER CUBIC MILLIMETER, AFTER SINGLE DOSES OF CORTISONE ACETATE AND AQUEOUS VEHICLE

(1) TEST MATERIAL	(2) TIME IN ZONES	(3) TIME IN HOURS	(4) NEUTRO-PHILS	(5) LYMPHO-CYTES	(6) EOSINO-PHILS	(7) TOTAL W.B.C.
		0	5,070	3,090	130	8,650
	1	1½	4,480	2,330	111	7,150
	2	3½	4,750	2,390	124	7,470
Aqueous vehicle	3	5½	5,960	2,780	126	9,140
intramuscularly	4	7½	6,740	2,610	101	9,770
	5	10½	5,710	2,880	80	8,910
	6	14½	5,340	3,040	87	8,690
	7	25	4,790	2,650	119	7,830
		0	4,410	2,730	116	7,580
	1	1½	5,010	2,380	112	7,670
	2	3½	5,940	1,970	78	8,210
Cortisone acetate	3	5½	7,620	1,860	59	9,700
orally	4	7½	7,660	2,240	49	10,160
	5	10½	5,450	3,030	71	8,800
	6	14½	5,430	3,240	99	9,020
	7	25	6,390	2,750	120	9,570
		0	4,940	2,770	130	7,820
	1	1½	4,930	2,420	116	7,670
	2	3½	5,500	2,480	95	8,390
Cortisone acetate	3	5½	7,720	2,620	103	10,100
intramuscularly	4	7½	10,740	2,600	106	14,070
	5	10½	10,700	2,790	61	13,740
	6	14½	10,200	3,120	81	13,620
	7	25	6,510	2,660	109	9,600

The eosinophil count declined after the intramuscular administration of the vehicle. A consistent decrease was first apparent at about seven and one-half hours after zero time (zone 4), and the count fell to a value of 80 cells per cubic millimeter at about ten and one-half hours (zone 5). There appeared to have been a gradual return toward the initial level by the following morning when the count was 119 cells per cubic millimeter.

The trend in the total white blood cell count reflected the summation of the changes in neutrophils, lymphocytes, and eosinophils. The relative effect of the neutrophil count was predominant, as would be expected from the proportional contribution of this cell type to the usual total white blood cell count.

B. *Oral Administration of Cortisone Acetate:* Administration of cortisone acetate orally was followed by an increase in the neutrophil count and by distinct declines in the lymphocyte and eosinophil counts.

The neutrophil count rose promptly and steadily from a level of 4,410 cells per cubic millimeter at zero time to a count of 7,660 cells per cubic millimeter seven and one-half hours later (zone 4). By ten and one-half hours after zero time (zone 5) the count had returned to about the initial level. This rise in the neutrophil count, during the first few hours after giving cortisone acetate orally, exceeded the corresponding rise which was observed after the intramuscular injection of aqueous vehicle.* However, the difference between the neutrophil counts, observed after these two test materials, was statistically significant† in zone 3 only.

The lymphocyte count decreased, after cortisone acetate was given orally, from an initial level of 2,730 cells per cubic millimeter to 1,860 cells per cubic millimeter at five and one-half hours after zero time (zone 3). This count had returned to a value of 3,030 cells per cubic millimeter by ten and one-half hours after treatment (zone 5). The lymphocyte counts at three and one-half hours and five and one-half hours (zones 2 and 3) were significantly lower than the corresponding counts after intramuscular administration of the vehicle.

The eosinophil count also showed a distinct decline after the oral dose of cortisone acetate. The lowest count was 49 cells per cubic millimeter at seven and one-half hours after zero time (zone 4), after which there was a gradual return toward the initial level of 116 cells per cubic millimeter. The fall in eosinophil count, after cortisone acetate orally, was significantly greater than that which followed the injection of vehicle at three and one-half, five and one-half, and seven and one-half hours after zero time (zones 2, 3, and 4, respectively).

The total white blood cell count reflected the net effect of the rise in neutrophil count and the fall in lymphocyte and eosinophil counts, and closely resembled the trend in the total count after intramuscular administration of the vehicle.

C. *Intramuscular Administration of Cortisone Acetate:* The most interesting of the fluctuations in white blood cell counts observed in this experiment was the marked rise in the neutrophil count which followed the intramuscular injection of 50 mg. of cortisone acetate. The neutrophil count increased from an initial value of 4,940 cells per cubic millimeter to 10,740 cells per cubic millimeter at seven and one-half hours after the injection (zone 4). This relatively high level was maintained beyond fourteen and one-half hours after zero time.

*One subject showed a greater rise in neutrophil count after the injection of aqueous vehicle than after the administration of cortisone acetate either orally or intramuscularly.

†The term "significant," as used in this paper, refers to statistical significance which the authors define as follows: for two test materials and a particular time zone, the mean of the differences between the two cell counts observed in each subject would be exceeded by chance less than 5 per cent of the time. The expected frequency of a chance difference was estimated under the hypothesis that, on the average, there would be no difference between the cell counts obtained after giving the two test materials.

The size and duration of the elevated neutrophil count exceeded the corresponding changes which followed the oral dose of cortisone acetate and the injection of aqueous vehicle. These differences were statistically significant at seven and one-half, eleven and one-half, and fourteen and one-half hours after zero time (zones 4, 5, and 6, respectively).

After cortisone acetate was given intramuscularly, the lymphocyte count showed only slight changes which closely resembled the trend observed after intramuscular injection of the vehicle.

The eosinophil count declined gradually from an initial value of 130 cells per cubic millimeter to 61 cells per cubic millimeter at about ten and one-half hours after zero time (zone 5), and afterwards returned toward the initial level. However, these changes were not significantly different from the corresponding counts observed after injection of the vehicle.

A significant increase in the total white blood cell count occurred after giving cortisone acetate intramuscularly. From an initial value of 7,820 cells per cubic millimeter, the total count rose to 14,070 cells per cubic millimeter at about seven and one-half hours after zero time (zone 4), and remained distinctly elevated beyond fourteen and one-half hours (zone 6). These changes clearly reflected the predominant effect of the increase in the neutrophil count.

II. *Subjective Responses.*—

A. *Intramuscular Administration of Aqueous Vehicle:* Seven of the eight subjects did not have any complaints after receiving the aqueous vehicle intramuscularly. One subject complained of moderate discomfort at the site of injection. His discomfort began about three hours after the injection and lasted about six hours. This same subject was referred to previously (footnote) as having shown a greater rise in neutrophil count after injection of the vehicle than after administration of the other two test materials.

B. *Oral Administration of Cortisone Acetate:* None of the subjects complained of any gastrointestinal or other symptoms after receiving the single dose of cortisone acetate orally.

C. *Intramuscular Administration of Cortisone Acetate:* All of the eight subjects experienced discomfort at the site of injection of cortisone acetate. The discomfort began about two to three hours after the injection, reached its peak of intensity eight to ten hours later and had subsided by the following morning. At no time were the symptoms severe enough to prevent the subjects from carrying on their normal activities.

SUMMARY

Single doses of 50 mg. of cortisone acetate orally and intramuscularly in healthy ambulatory subjects were followed by different and distinct patterns of behavior of the neutrophil, lymphocyte, eosinophil, and total white blood cell counts of the peripheral blood.

When compared with the fluctuations in cell counts after an intramuscular injection of the aqueous vehicle, in which cortisone acetate is suspended for intramuscular use, the oral administration of cortisone acetate resulted in an

increase in the neutrophil count during the first seven and one-half hours after the drug was given. There were also significant decreases in the lymphocyte and eosinophil counts after cortisone acetate orally, but no relative change in the total white blood cell count.

In contrast to these results, the intramuscular injection of cortisone acetate was followed by a significantly greater and more prolonged increase in the neutrophil count and a significant rise in the total white blood cell count, but no appreciable changes in the lymphocyte and eosinophil counts. In addition, the rise in the neutrophil and total white blood cell counts, after cortisone acetate was given intramuscularly, was roughly paralleled in time and magnitude by discomfort at the site of injection in all subjects.

CONCLUSIONS

These observations lead to the following conclusions:

In healthy ambulatory subjects a single oral dose of 50 mg. of cortisone acetate is followed by an increase in the neutrophil count and decreases in the lymphocyte and eosinophil counts, during the first few hours after giving the drug.

In healthy ambulatory subjects a single intramuscular dose of 50 mg. of cortisone acetate is followed by a marked and prolonged increase in the neutrophil and total white blood cell counts which persists for more than 14 hours, and these changes are related in time and magnitude to discomfort at the site of injection. There are no significant changes in the lymphocyte and eosinophil counts.

It is postulated that the prompt changes in white blood cell counts after an oral dose of cortisone acetate are specific pharmacological effects of the drug, but that those changes which occur after an intramuscular dose of cortisone acetate are due not only to pharmacological effects but in part to mechanical irritation at the site of injection.

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*Since this article was accepted for publication, the authors' attention has been drawn to the recently published work of Nelson, D. H., Sandberg, A. A., Palmer, J. G., and Tyler, F. H.: Blood levels of 17-Hydroxycorticosteroids Following the Administration of Adrenal Steroids and Their Relation to Levels of Circulating Leukocytes, J. Clin. Investigation **31**:843-849, 1952. These investigators observed changes in the white blood cell counts of normal human subjects after administration of cortisone acetate orally and intramuscularly which are similar to the findings in the present study.

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THE DIFFERENTIAL RATES OF DIFFUSION OF NITROUS OXIDE INTO THE CEREBROSPINAL FLUID

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WHILE investigating the effect of the diffusion of nitrous oxide into the cerebrospinal fluid on the determination of cerebral blood flow by the nitrous oxide technique, it was observed that the concentration of the gas rose at different rates in the ventricular, cisternal, and lumbar cerebrospinal fluid compartments.[1] Further investigation of this phenomenon was undertaken with the hope that it might present additional data regarding the problem of the origin of the cerebrospinal fluid.

METHOD

The subjects employed were under 40 years of age, had sustained traumatic head injuries one to three months prior to the investigation, and were in a state of irreversible post-traumatic coma. Blood and cerebrospinal fluid samples were drawn at various intervals while the patients were breathing a standard gas mixture† containing 15 per cent nitrous oxide, 21 per cent oxygen, and 64 per cent nitrogen. Blood samples were taken for analysis from the internal jugular vein and the femoral artery; samples of cerebrospinal fluid were obtained from a lateral cerebral ventricle (through a trephine opening made one to three months prior to this investigation), the lumbar sac, and the subarachnoid space at the base of the brain through the jugular foramen. The technique for obtaining the latter sample has been described in a previous communication.[1] Cerebral blood flow was determined according to the method devised by Kety and Schmidt[2] and modified by Scheinberg and Stead.[3] Nitrous oxide determinations were done as previously described.[2]

In patient No. 1 and patient No. 2, the arterial and venous blood, and ventricular and cisternal cerebrospinal fluid samples were withdrawn simultaneously at two and one-half, five, seven and one-half, ten, fifteen, and twenty-five minutes of inhalation of the gas mixture. The cerebral blood flow on these patients had been determined less than two weeks prior to the above studies. In patient No. 3 and patient No. 4, arterial and cerebral venous blood samples were withdrawn continuously and simultaneously during the first ten minutes of inhalation of the gas mixture, and the cerebral blood flow was determined. In these two subjects, ventricular and cisternal cerebrospinal fluid samples were also withdrawn simul-

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†Used for the determination of cerebral blood flow. Vide infra.

taneously every five minutes over a thirty-minute period of gas inhalation. In patient No. 3, a repeat cerebral blood flow study was done the following week, at which time lumbar subarachnoid cerebrospinal fluid samples were withdrawn every five minutes for a thirty-minute period. In patient No. 4, repeat studies were done two weeks after the initial study, at which time lumbar and cisternal cerebrospinal fluid samples were withdrawn simultaneously at five-minute intervals over a thirty-minute period of gas inhalation, and the rate of cerebral blood flow was again determined.

RESULTS

The rates for cerebral blood flow and the concentration of nitrous oxide in the ventricular, cisternal, and lumbar cerebrospinal fluid and arterial and cerebral venous blood over a twenty-five to thirty-minute period are presented in Table I. Fig. 1 compares graphically the rate of appearance of nitrous oxide in the ventricular, cisternal, and lumbar cerebrospinal fluid in all cases, and Fig. 2 presents the average rates of appearance of the gas into each of these compartments. The data demonstrate that the nitrous oxide content of the cisternal cerebrospinal fluid consistently rose more rapidly than that of either of the other two cerebrospinal fluid compartments. In no case did the rate of appearance of nitrous oxide in the ventricular fluid equal the rate of appearance of the gas in the cisternal cerebrospinal fluid, i.e., the lowest rate

TABLE I. BLOOD AND CEREBROSPINAL FLUID NITROUS OXIDE CONTENT OVER TWENTY-FIVE TO THIRTY-MINUTE INHALATION OF 15 PER CENT NITROUS OXIDE

CASE	MIN.	DIAGNOSIS	CBF	ART.	VEIN	CIST.	VENT.	LUMB.
1	2½	Traumatic		3.69	1.56	1.30	0.03	
	5	Coma		4.26	2.98	2.34	0.27	
	7½	Age 39		4.33	3.55	2.72	0.58	
	10		30	4.58	3.88	2.75	0.95	
	15			4.49	4.23	3.39	1.51	
	25			4.69	4.69	3.73	2.69	
2	2½	Traumatic		3.66	2.02	0.06	0.07	
	5	Coma		4.01	2.84	0.48	0.07	
	7½	Age 36		4.43	3.37	0.88	0.05	
	10		28	4.55	3.67	1.36	0.06	
	15			4.73	4.13	1.95	0.16	
	25			5.23	4.72	2.81	0.55	
3	5	Traumatic				0.37	0.02	0.20
	10	Coma	25	4.73	3.54	1.16	0.02	0.24
	15	Age 25				1.78	0.13	0.26
	20					2.36	0.26	0.43
	25					2.19	0.38	0.74
	30							0.95
4	5	Traumatic				1.07	0.00	0.28
	10	Coma	33	4.43	4.29	2.19	0.21	0.69
	15	Age 22				2.79		
	20					3.34	1.54	
	25					3.61	2.20	2.03
	30					3.92	2.37	2.38

Min. = Minutes of inhalation of 15 per cent nitrous oxide.
CBF = Cerebral blood flow (in ml. blood/100 Gm. brain/min.).
Art. = Arterial nitrous oxide, volumes per cent.
Vein = Internal jugular venous nitrous oxide, volumes per cent.
Cist. = Intracranial subarachnoid ("cisternal") cerebrospinal fluid nitrous oxide, volumes per cent.
Vent. = Intraventricular cerebrospinal fluid nitrous oxide, volumes per cent.
Lumb. = Lumbar cerebrospinal fluid nitrous oxide, volumes per cent.

of appearance of nitrous oxide in the cisternal fluid was faster than the most rapid rate of appearance in the ventricular fluid. When the rates of increase of nitrous oxide content in the ventricular and cisternal fluids are computed from the rapidly rising section of the curve (Fig. 1), it appears that nitrous oxide rises from two to five times as fast in the latter as in the former. Thus, a definite relationship between the nitrous oxide levels of the two compartments exists.

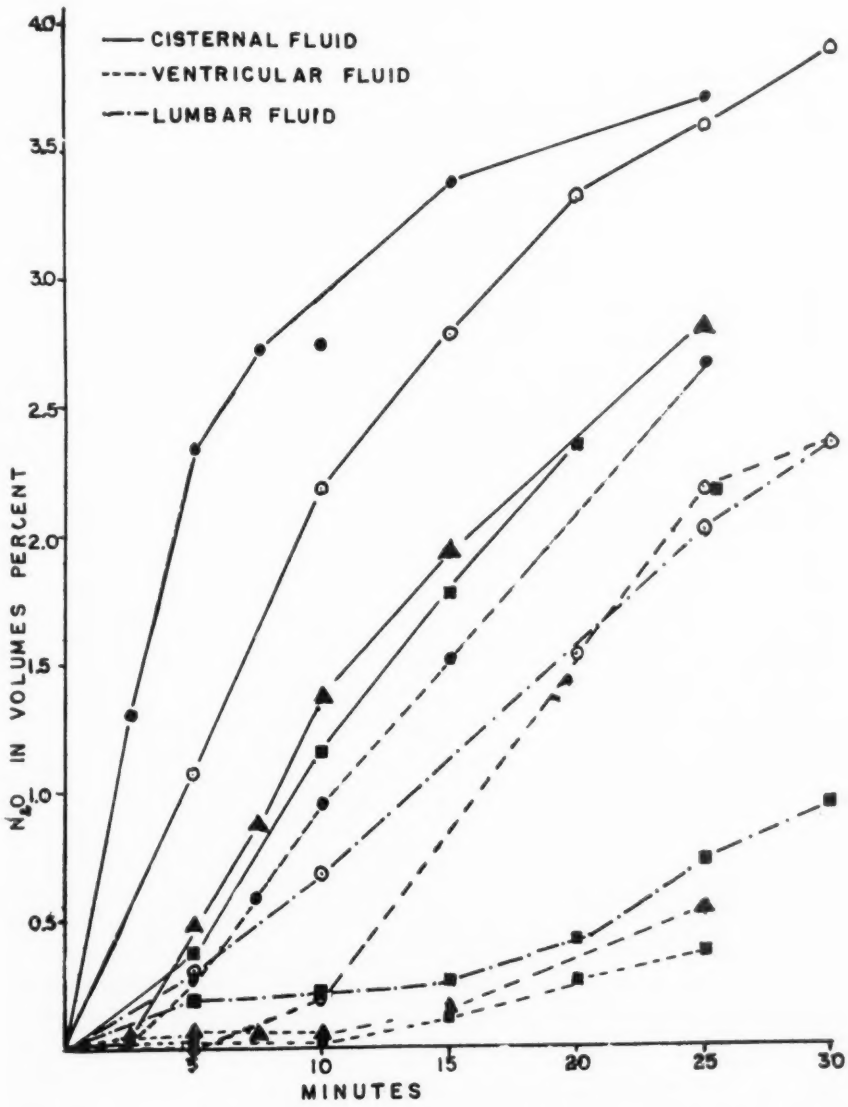

Fig. 1.—Rate of appearance of nitrous oxide in ventricular, cisternal, and lumbar cerebrospinal fluid in all cases. Case 1, ●; Case 2, ▲; Case 3, ■; and Case 4, ⊙.

The rate of appearance of nitrous oxide into the lumbar sac fluid roughly approximates that of the ventricular. In one case, the lumbar cerebrospinal fluid curve is slightly higher than the ventricular curve (No. 3); and in the other, the lumbar cerebrospinal fluid nitrous oxide content rose faster initially but the ventricular was slightly higher at the end of thirty minutes (No. 4). In both of these patients the relationship between the curves of the three various compartments is the same. In Fig. 2, which illustrates the average rates of entrance of nitrous oxide into cerebrospinal fluid, the curve obtained for lumbar cerebrospinal fluid appears only slightly higher than that for ventricular fluid.

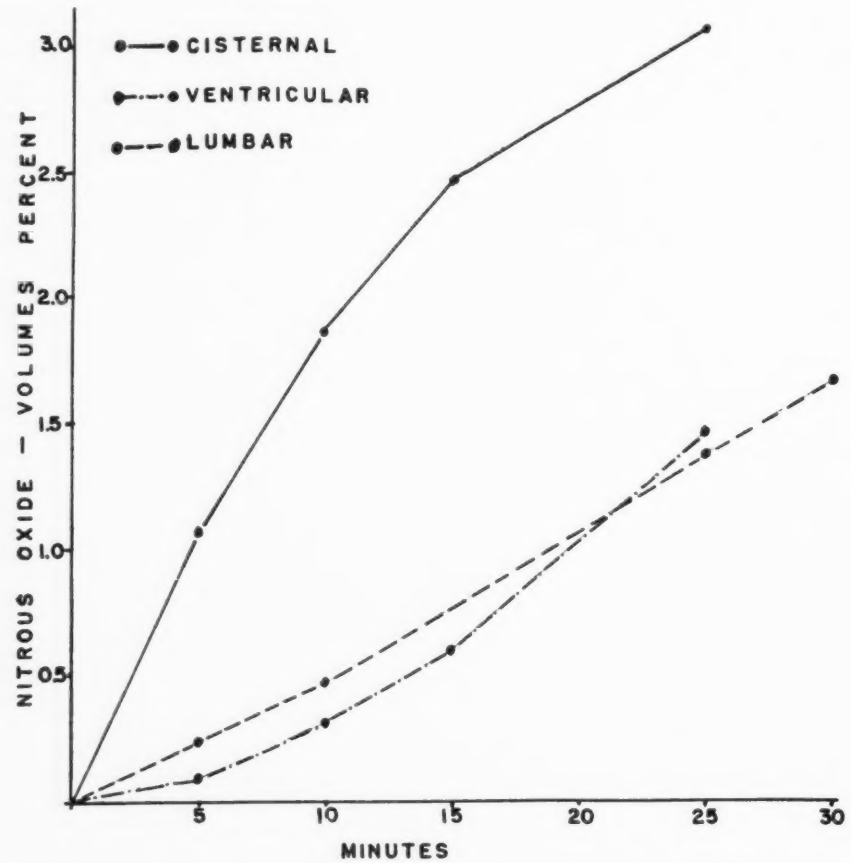

Fig. 2.—Average rate of appearance of nitrous oxide in ventricular, cisternal, and lumbar cerebrospinal fluid.

At the time of the second study on patient No. 4, when simultaneous lumbar and cisternal cerebrospinal fluid samples were withdrawn, the lumbar fluid was xanthochromic, while the cisternal fluid was clear. Subsequent analysis revealed that the lumbar fluid protein was 220 mg. per cent and the

cisternal fluid protein was 17 mg. per cent. Partial block between the lumbar sac and cistern was postulated. The nitrous oxide concentration in the lumbar fluid of this patient nevertheless rose as rapidly (in relation to the relative rates of increase of the gas in the cisternal and ventricular fluid) as that of the patient without a block, while the absolute nitrous oxide content actually rose faster in this patient's lumbar fluid than in that of the patient without evidence of a block.

The data also indicate a definite relationship between the rates of appearance of nitrous oxide into the various compartments. Patient No. 1 who had attained the highest level of nitrous oxide in the cisternal fluid also exhibited the highest level in the ventricular fluid. Conversely, the two patients whose cisternal fluid nitrous oxide content rose most slowly also demonstrated the slowest rates of increase of the gas in the ventricular fluid. These two patients (Nos. 2 and 3) showed practically identical levels of nitrous oxide concentration in both the cisternal and ventricular cerebrospinal fluid samples.

The rates of cerebral blood flow was abnormally low in all cases. Other studies have shown that the cerebral blood flow in irreversible traumatic coma is consistently reduced below normal values.[4] However, there is no evident correlation in the subjects presented between the rate of cerebral blood flow and the concentration of nitrous oxide in the cerebrospinal fluid of the various compartments.

DISCUSSION

Since the studies of Flexner,[5] Dandy and Blackfan,[6] Weed,[7] and others,[8, 9] the belief has been widely held that the choroid plexus is the chief, if not the only site of formation of cerebrospinal fluid. However, there are some[10, 11] who are of the opinion that the choroid plexus plays a minor role in the formation of cerebrospinal fluid and that this fluid is more a reflection of the extracellular tissue fluid surrounding the cerebral cells. There is anatomic evidence which suggests that the perivascular spaces of Virchow-Robin may participate to some degree in this process by providing communication between the subarachnoid cerebrospinal fluid and the extracellular tissue fluid of the cerebral cells.[12, 13]

In 1925, Cestan, Laborde, and Riser[14] injected sodium salicylate and urea into the saphenous vein of dogs, and a short time thereafter removed simultaneous samples of lumbar and cisternal cerebrospinal fluid. The concentrations of the injected substances were always as great in the lumbar as in the cisternal cerebrospinal fluid. Appreciable concentrations of the test substance appeared in the lumbar spinal fluid when the lumbar sac was separated from the more superior portions of the cerebrospinal fluid system by ligature prior to the injection of the test substance. In 1940, Wallace and Brodie[11] conducted similar experiments injecting bromides and iodides both intravenously and intracisternally. They concluded that the chief site of entrance of these anions, as

evidenced by their distribution throughout the central nervous system, is not through the choroid plexus but that they pass from the circulating blood into the extracellular tissue fluid and from here spread out through the subarachnoid fluid. In 1949, Bakay and Lindberg[15] injected P^{32} intracisternally in rabbits and concluded that some minor portions of the brain are supplied with phosphorus through the blood (i.e., choroid plexus, hypophysis, pineal and vegetative centers in the hypothalamus), but that most of the nervous tissue "seems to exchange phosphate via the cerebrospinal fluid." The concentration of intravenously injected radioactive isotopes in the cerebrospinal fluid of a 4-year-old child with a complete block between the ventricles and the lumbar sac (tumor of the third ventricle) was recently studied by Boldrey and associates. From their data, the authors concluded that there is formation of fluid both in the ventricles and the spinal subarachnoid space.[16] Sweet and associates[17] studied the rate of appearance of intravenously injected Na^{24} and heavy water in the three cerebrospinal fluid compartments in man. The Na^{24} rose most rapidly in the ventricular fluid, second in the cisternal, and most slowly in the lumbar, while heavy water rose most rapidly in the cisternal fluid, second in the ventricular, and most slowly in the lumbar. They concluded that "the choroid plexus does not appear to be necessary to the exchange of water between blood and cerebrospinal fluid."

Our findings indicate that nitrous oxide may also gain access to cerebrospinal fluid by a route other than the choroid plexus. If spinal fluid was formed chiefly by the choroid plexus, the ventricular fluid nitrous oxide concentration would be expected to rise more rapidly and to attain the highest levels, while the level of the gas in the lumbar sac fluid should rise more slowly, since diffusion from above (i.e., from the ventricular fluid) would be its only source of entry. The intracranial subarachnoid spinal fluid nitrous oxide concentration should rise somewhat more rapidly than the lumbar fluid but more slowly than the ventricular. However, in our study the cisternal cerebrospinal fluid nitrous oxide levels without exception rose most rapidly and to the highest levels, while the ventricular and lumbar fluid gas concentrations were of the same order of magnitude.

If nitrous oxide enters the subarachnoid cerebrospinal fluid by diffusion into the Virchow-Robin spaces (perhaps across the membrane separating blood from cerebrospinal fluid), one would expect the nitrous oxide concentration to be highest in the cisternal fluid for the following reasons: first, the Virchow-Robin spaces drain only into the subarachnoid fluid and not into the ventricular fluid.[17] Therefore, of the three sampled compartments, only the lumbar and cisternal fluids would receive contributions directly from these spaces. Second, the amount of nitrous oxide in the subarachnoid fluid would be expected to bear some relationship to the surface area available to this fluid for diffusion to take place. By far the largest surface area of the brain substance is that of the cortex, which, including the convolutions and gyri, has been estimated at 2,000 square centimeters.[18] The additional surface area of the pons, medulla, and cerebellum is no more than 400 square centimeters. The surface area of the

spinal cord can be roughly approximated at 240 square centimeters.* Therefore, the total surface area available for diffusion of nitrous oxide into the intracranial subarachnoid spinal fluid is roughly ten times that of the extracranial spinal fluid. If the mechanism by which nitrous oxide reaches both compartments is the same (as postulated), the concentration of nitrous oxide in the lumbar fluid should be roughly one-tenth of that of the cisternal fluid. The values obtained in this study approximate this relationship (Table I). One should take into consideration also the large area represented by the interfaces between the capillaries and their Virchow-Robin spaces. Unfortunately, quantitative data on the size of such a vascular bed are not available. Whether or not a similar correlation exists between the diffusion gradients and these interfaces cannot be ascertained at this time.

The source of the nitrous oxide which appears in the ventricular fluid is not clear. No doubt some of it comes through the choroid plexus. In addition, there are certain areas of the brain (area postrema of the hindbrain, the supraoptic crest of the diencephalon, and the infundibulum) which resemble each other (thus differing from the rest of the brain) in the richness and complexity of their blood supply.[20] Since the supraoptic crest is adjacent to the ventricle, some of the nitrous oxide may diffuse directly from the blood stream of this vascular area of the brain into the ventricle.

The ventricular cerebrospinal fluid curves show a lag from two and one-half to ten minutes before the curves (Fig. 1) show a rapid rise. Those two patients (No. 2 and No. 3) in whom there was a short lag (two and one-half minutes) in the cisternal curves showed a prolonged lag in the ventricular curve. Patients No. 1 and No. 4, who showed no lag whatsoever in the cisternal curve, showed a shorter lag in the ventricular curve. At all times there was a substantial quantity of nitrous oxide in the intracranial subarachnoid fluid before any significant amount entered the ventricular fluid. This finding eliminates the possibility that nitrous oxide originates in the ventricle and passes to (perhaps accumulating in) the cisternal fluid. It is also possible that much of the nitrous oxide content of the ventricular fluid comes from the subarachnoid fluid as it circulates through the ventricular system.

The initial segment of the lumbar curves, on the other hand, shows no such lag. Here there is an initial rapid rise with a later more gradual rate of increase. Such findings imply that the rapidly rising component is due to diffusion of the nitrous oxide from the lumbar cord itself; whereas the latter more slowly rising component may be due to mixing (artificial and otherwise) with the intracranial subarachnoid fluid. (Vide infra.)

*The average length of the spinal cord and the average diameters in the lumbar, thoracic, and cervical areas are known.[19] Assuming that the spinal cord is an ellipse, the formula for the perimeter of an ellipse may be applied for each area.

$$P = \pi (A + B)\left[1 + \left(\tfrac{1}{4}\left(\frac{A - B}{A + B}\right)^2 + 1/64\left(\frac{A - B}{A + B}\right)^4\right)\right]$$

The additional length of the anterior fissure (one-third the narrow dimension) must be added for each area. The total figure thus obtained is multiplied by the average length of each segment and the sum of the figures thus obtained equals an approximation of the surface area of the cord. Since the spinal cord is not a perfect ellipse, this is obviously only a rough calculation.

It should be pointed out that when samples of cerebrospinal fluid are withdrawn as described in the method, an artificial circulation of the fluid may be produced. As samples of cerebrospinal fluid are being withdrawn simultaneously from the ventricular and cisternal spaces, currents of flow thus produced would be in opposite directions and tend to neutralize each other in regard to drawing ventricular fluid into the cisternal space, and vice versa. It is our feeling, therefore, that the ventricular fluid is relatively uncontaminated by cisternal fluid and vice versa as a result of the simultaneous withdrawal of samples. In corroboration of this line of reasoning, independently drawn cisternal fluid samples (patient No. 4, data not included on table) showed a decreased nitrous oxide curve (dilution with ventricular fluid), while separately drawn ventricular samples showed increased nitrous oxide content and no initial lag in the curve (contamination from cisternal fluid). The possibility of the artificially induced currents produced by the withdrawal of fluid, causing an abnormally rapid mixing of the cerebral extracellular fluid with the cerebrospinal fluid of the spaces of Virchow-Robin and ultimately with the cerebrospinal fluid being samples in either the cerebral subarachnoid or lumbar spaces, is not precluded.

In addition, it must be remembered that the patients studied were in a state of irreversible post-traumatic coma and had been trephined (one to three months prior to the study) for possible subdural hematomas. One cannot dogmatically state that similar diffusion gradients would obtain for normal subjects. However, since analogous curves were obtained in conscious patients,[17] we feel that the clinical condition of these patients did not significantly alter the method in which nitrous oxide was handled by the central nervous system.

We have assumed that the process by which the nitrous oxide gains entrance to the cerebrospinal fluid is by diffusion. (However, the possibility of a secretory mechanism cannot be eliminated by the data at hand.) It may further be argued that nitrous oxide is a gas and as such is subject to different laws of diffusion than those for the normal constituents of cerebrospinal fluid. Kety and associates[22] have shown that the blood-brain partition coefficient of nitrous oxide is only slightly removed from unity, despite the greater solubility of nitrous oxide in common fats and oils over that in water or blood. Thus, the diffusion of the gas would not be influenced by lipid concentration in various portions of the brain. However, whatever the mechanism, it is probable that nitrous oxide moves with water[17] as it enters the cerebrospinal fluid, since both of these substances show maximum rates of increase in the cisternal rather than the ventricular fluid.

SUMMARY AND CONCLUSIONS

The rates of diffusion of nitrous oxide into the ventricular, cisternal, and lumbar cerebrospinal fluid over a thirty-minute period have been measured.

The concentration of nitrous oxide in the cisternal fluid rises more rapidly than in either the lumbar or ventricular cerebrospinal fluid. The latter show rates of diffusion of essentially the same order of magnitude. An initial lag is seen in the ventricular curve only.

Nitrous oxide may enter the cerebrospinal fluid via routes other than the choroid plexus. Possible modes of entry are discussed.

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LIPASE IN HUMAN BLOOD PLASMA

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THE presence of lipase in the plasma has been a controversial subject. Bloor[1] refers to the work of Hanriot published in 1898, according to which true lipase is present in blood. On the other hand, Noguchi[2] was of the opinion that since the enzyme did not hydrolyze higher glycerides, true lipase could not exist as such in blood. Only recently, Comfort and Osterberg[3] have demonstrated the occurrence of an enzyme in the serum of human subjects capable of splitting the glycerides of olive oil. Goldstein, Epstein, and Roe[4] have estimated the lipolytic activity of plasma using tributyrin as substrate. The method employed by them cannot, however, be considered as specific for lipase, for both esterase and lipase can hydrolyze tributyrin under the conditions used by them. Recently, Seligman and Nachlas,[5] using the chromogenic substrate napthyl laurate and taking advantage of differences in the action of sodium taurocholate on esterase and lipase, concluded that true lipase was not present in the human blood serum. Their conclusion was based on the observation that sodium taurocholate inhibited esterase and stimulated lipase. Since, however, the inhibition of esterase action on their substrates was only partial, it is possible that the activation of lipase by the taurocholate may have cancelled the effect due to the inhibition of esterase, or the latter may have exceeded the activation of lipase. In the likelihood of the occurrence of lipase and esterase together, the activity of the mixture obtained with or without sodium taurocholate would be identical in the former case, whereas in the latter, a decrease in activity should result with sodium taurocholate. In either case, the result should be inconclusive. So it was felt that the use of a substance which completely inhibited only one of the two enzymes should yield a more conclusive proof for the presence or absence of a lipase in plasma. The present work demonstrates the occurrence of lipase in the plasma by the utilization of such a technique.

In order to evolve such a method, it was necessary to study the relationship of certain enzyme inhibitors and accelerators on both esterase and lipase separately and together. For this purpose preparations of esterase and lipase from beef liver and pancreas, respectively, were used.

During the course of the experiments, it was found that whereas the activity of lipase was enhanced by sodium tauroglycocholate, it completely blocked the action of esterase on tributyrin. This observation has been utilized as the basis of differentiation between esterase and lipase.

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MATERIALS AND METHODS

Liver esterase.—The procedure employed for the preparation of liver esterase was based on the method used by Willstätter and Memmen.[6] Beef liver was extracted with twice its volume of 1 per cent (w/v) ammonia. The esterase present in the ammonia extract was adsorbed over acid-washed kieselguhr at pH 4.2, and eluted with the aid of dilute ammonia. To the eluate was added ammonium sulfate to full saturation, the precipitate formed was collected, washed with acetone, and dried in vacuo. For the estimation of its activity, the powder was shaken with water and centrifuged. The centrifugate was dialysed against distilled water overnight, centrifuged, and the clear supernatant containing the enzyme in solution was used.

Kidney esterase.—Beef kidney was extracted with 1 per cent (w/v) ammonia and the supernatant fluid obtained after centrifugation was adjusted to pH 7.0 and employed as the source of esterase.

Pancreatic lipase.—Beef pancreas freed from fibrous tissue was extracted with large quantities of 1:1 acetone-ether mixture and dried in vacuo. The dry powder was extracted with 87 per cent aqueous glycerol. The extract was centrifuged; the supernatant after appropriate dilution served as the source of lipase.

Estimations.—Esterase was determined by the method of Cherry and Crandall[7] using ethyl butyrate as substrate at 0.1M concentration. The acid liberated was titrated against N/20 sodium hydroxide.

Lipase activity was measured by the method of Goldstein, Epstein, and Roe[4] with slight modifications. The substrate was made up by dispersing 2.9 ml. of tributyrin in 50 ml. of 2 per cent solution of calcium acetate and 50 ml. of a 0.5 per cent solution of sodium barbiturate. A small amount of gum acacia (200 mg./100 ml.) was added to facilitate dispersion. To 10 ml. of the substrate was added the necessary amount of the enzyme, and the acid liberated on incubation at 37° C. for different periods was titrated with N/20 sodium hydroxide. A control tube containing "boiled enzyme" was run under the same conditions to obtain the blank value.

RESULTS

The activity of each of the two enzymes, viz., pancreatic lipase and liver esterase, was tested against the two substrates—ethyl butyrate and tributyrin. Table I summarizes the results obtained.

TABLE I. ESTEROLYTIC ACTIVITIES OF LIVER ESTERASE AND PANCREATIC LIPASE UPON ETHYL BUTYRATE AND TRIBUTYRIN

ENZYME	HOURS OF INCUBATION	TITER VALUE (ML. N/20 NaOH)	
		ETHYL BUTYRATE	TRIBUTYRIN
Liver esterase	4	0.95	0.8
	8	1.75	1.9
	24	2.50	3.5
Pancreatic lipase	4	0	5.7
	8	0	8.7
	24	0	10.5

From the results, it may be seen that liver esterase is capable of splitting both the substrates, whereas pancreatic lipase is inactive towards ethyl butyrate. The concentration of enzymes employed in the experiments was kept low. The esterase activity of normal human plasma estimated by the method of Cherry and Crandall[7] rarely, if ever, exceeded a value of 180 units per 100 ml. of plasma. The concentration of esterase used in the above experiment was sufficient to give a value of 240 units per 100 ml. Since the object of this work was to find conditions applicable to plasma, low concentrations of enzymes simulating those obtained in plasma had to be used in the experiments.

TABLE II. INFLUENCE OF SODIUM CETYL SULFATE (0.5×10^{-3} M) ON ESTERASE AND LIPASE ACTION WITH TWO SUBSTRATES

	HOURS OF INCUBATION	TITER VALUE (ML. N/20 NaOH)	
		ETHYL BUTYRATE	TRIBUTYRIN
Trials with esterase			
Esterase alone	4	1.00	0.85
	8	2.00	2.00
	24	3.00	3.70
Esterase + sodium cetyl sulfate	4	0	0
	8	0	0
	24	0	0
Trials with lipase			
Lipase alone	4	0	5.7
	8	0	8.65
	24	0	10.50
Lipase + sodium cetyl sulfate	4	0	0
	8	0	0
	24	0	0

Eserine, quinine, sodium arsenilate, sodium cetyl sulfate, and sodium taurocholate, among others, have been widely used for their effects upon the esterolytic activity of tissue extracts. In the present investigation, only the last two have been used.

Sodium cetyl sulfate.—Schulman[8] had observed that sodium cetyl sulfate inactivated lipase almost comp'etely. Frazer[9] had successfully used this reagent to inhibit lipolysis in the intestines in his studies on particulate absorption of fat. It was, therefore, felt that pancreatic lipase could be differentiated from esterase provided the latter was unaffected by sodium cetyl sulfate. Trials with different concentrations of sodium cetyl sulfate and the two enzymes showed, however, that the substance inactivated not only lipase but esterase as well. In Table II are given the results obtained with the lowest concentration of cetyl sulfate which brought about complete inhibition of both of the enzymes.

Sodium tauroglycocholate.—Willstätter and Memmen[6] observed an accelerating effect of sodium taurocholate upon the hydrolysis of methyl butyrate and triacetin by pancreas at a concentration of 2×10^{-2} M. This was supported by the findings of Gomori,[10] who noted an increased staining of pancreatic tissue with sodium taurocholate in his histochemical method, although he also observed some inhibition of liver and kidney staining. According to Nachlas and Seligman,[11] the activity of kidney and liver esterases was slightly inhibited, whereas the pancreatic activity was markedly accelerated with sodium taurocholate. Holwerda[12] has determined the optimum concentration of sodium glycocholate for the activation of pancreatic lipase, and he concludes that the glycocholate acts by aiding the union of enzyme with the fat. In our experiments sodium tauroglycocholate at a concentration of 3.7×10^{-3} M was used. The results are outlined in Table III.

It is evident that sodium tauroglycocho'ate activates lipase, but it was interesting to find liver esterase being completely inhibited by it when tributyrin was used as substrate. On the other hand, esterase activity toward ethyl butyrate was only partially inhibited. This observation, one felt, could be utilized as the basis of differentiation between esterase and lipase when the two

TABLE III. INFLUENCE OF SODIUM TAUROGLYCOCHOLATE (3.7×10^{-3} M) ON ESTERASE AND LIPASE ACTION WITH TWO SUBSTRATES

	HOURS OF INCUBATION	TITER VALUE (ML. N/20 NaOH)	
		ETHYL BUTYRATE	TRIBUTYRIN
Trials with esterase			
Esterase alone	4	0.95	0.85
	8	1.80	1.80
	24	2.40	3.20
Esterase + sodium tauroglycocholate	4	0.45	0
	8	0.65	0
	24	0.75	0
Trials with lipase			
Lipase alone	4	0	5.7
	8	0	8.7
	24	0	10.5
Lipase + sodium tauroglycocholate	4	0	6.5
	8	0	9.5
	24	0	11.3

enzymes coexist as they probably do in plasma. With tributyrin as substrate, the activity could be due either to esterase or lipase, or to both. However, the introduction of sodium tauroglycocholate into the reaction medium could alter the picture by completely inhibiting esterase action. Under these circumstances, only lipase activity would be indicated.

Before proceeding to test the human plasma for the presence of lipase, experiments were carried out to evaluate the action of sodium tauroglycocholate on an artificial mixture of esterase and lipase. The findings are summarized in Table IV.

TABLE IV. EFFECT OF SODIUM TAUROGLYCOCHOLATE (3.7×10^{-3} M) ON A MIXTURE OF ESTERASE AND LIPASE WITH SUBSTRATE TRIBUTYRIN

	TITER VALUE (ML. N/20 NaOH)
Esterase alone	1.20
Esterase + sodium tauroglycocholate	0
Lipase alone	2.90
Lipase + sodium tauroglycocholate	3.40
Esterase + lipase + sodium tauroglycocholate	3.35

As expected, the activity of esterase was completely inhibited and that of lipase enhanced by sodium tauroglycocholate. In the reaction mixture containing tauroglycocholate and the two enzymes only lipase activity was evident, as can be seen from the agreement between the last two values in column 2 of Table IV.

Using these observations as the basis, several specimens of human plasma were investigated for their activities on tributyrin as substrate, with and without sodium tauroglycocholate. From Table V it may be seen that in every case the plasma was active in the presence of sodium tauroglycocholate (column 3). This clearly demonstrates the existence of a lipase in the plasma, for this activity could be brought about only by lipase. The value obtained without sodium tauroglycocholate gives the combined activities of lipase and esterase.

DISCUSSION

The concentration of enzymes employed by us in the inhibition experiments is comparable with the amounts available in human plasma. Since in every

TABLE V. ESTEROLYTIC ACTIVITY OF NORMAL HUMAN PLASMA UPON SUBSTRATE TRIBUTYRIN, WITH AND WITHOUT SODIUM TAUROGLYCOCHOLATE (3.7×10^{-3} M)

NO.	WITHOUT SODIUM TAUROGLYCOCHOLATE* (UNITS/100 ML.)	WITH SODIUM TAUROGLYCOCHOLATE† (UNITS/100 ML.)
1	320	255
2	345	245
3	300	260
4	320	265
5	330	285
6	360	260
7	265	215
8	300	210
9	335	255
10	270	185
11	345	225
12	390	240

*The activity obtained gives the combined activities of both esterase and lipase.
†The activity obtained represents the accelerated activity of true lipase.

case of human plasma tested by us activity was obtained, it is but reasonable to conclude that an enzyme similar to that of pancreatic lipase occurs in plasma in addition to an esterase.

Seligman and Nachlas[5] have attributed the small amount of hydrolysis of olive oil obtained by the method of Cherry and Crandall[7] in normal serum to esterase. Roe and Goldstein,[13] and some others,[14, 15] have observed that liver does not produce an enzyme which splits the lipids of olive oil. In their work on cats, the former workers found, consequent to chloroform poisoning, an increase in the enzymes capable of splitting the substrates ethyl butyrate, tributyrin, and benzyl butyrate. On the other hand, the enzymes catalyzing the hydrolysis of triolein and olive oil slightly decreased. If the enzyme hydrolysing the lipids of olive oil were an esterase, there should have been an increase in olive oil splitting capacity of the blood, but this was not their experience. Comfort and Osterberg[3] have reported, as mentioned earlier, the presence in plasma of an enzyme capable of splitting the lipids of olive oil. These results lend support to our findings of the presence of a lipase in the plasma.

The source of plasma esterase is still not clear. Its possible origin in the liver is indicated by the work of Jobling and associates,[16] who found an increase in the plasma esterase after phosphorus and chloroform poisoning while the liver esterase diminished. Whipple[17] has suggested the possible use of plasma esterase determination as a measure of liver damage. Preliminary experiments by us did not reveal a difference between the plasma esterase activity in rats receiving injections of carbon tetrachloride and that of control animals, although in these animals the liver esterase activity decreased subsequent to liver damage.

The results reported in this paper were obtained by using liver esterase as the typical esterase. Since esterase occurs in several tissues, the possible differences in their behavior towards tauroglycocholate had to be taken into account. It was, therefore, considered desirable to study the action of kidney esterase on tributyrin under the same conditions as were used in liver esterase experiments. The results obtained (Table VI) were similar to those with liver esterase, and

TABLE VI. INFLUENCE OF SODIUM TAUROGLYCOCHOLATE (3.7×10^{-3} M) ON KIDNEY ESTERASE WITH SUBSTRATE TRIBUTYRIN

	HOURS OF INCUBATION	TITER VALUE (ML. OF N/20 NaOH)
(a) Kidney esterase alone	4	0.8
Kidney esterase + sodium tauroglycocholate	4	0
(b) Kidney esterase alone	4	1.2
Kidney esterase + sodium tauroglycocholate	4	0

hence it can be expected that esterase from other sources would also behave in a like manner. Thus plasma esterase, irrespective of its source, would probably be completely inhibited by sodium tauroglycocholate with tributyrin as substrate.

Based on the above results, the following method has been adopted for the estimation of lipase in the plasma. To 10 ml. of the substrate, prepared as described under "estimation," are added 0.5 ml. of sodium tauroglycocholate (3.7×10^{-3} M) and 0.5 ml. of plasma. The reaction mixture is incubated for four hours at 37° C. The acid liberated at the end of the period is titrated against N/20 sodium hydroxide using phenolphthalein as indicator. The blank value is assessed by running 0.5 ml. of "heat inactivated plasma" under the same conditions. The difference between the two gives the amount of lipase activity.

One lipase unit is the amount of enzyme necessary to catalyze the hydrolysis of tributyrin under the above conditions to yield a titer value of 1 ml. of N/10 sodium hydroxide.

SUMMARY

The presence of lipase in the plasma has been demonstrated by taking advantage of the inhibitory action of sodium tauroglycocholate on esterase-tributyrin system.

Since lipase was found not to hydrolyze ethyl butyrate, the latter could be safely used for the evaluation of esterase activity in plasma.

Sodium cetyl sulfate at a concentration of 0.5×10^{-3}M, completely inhibited liver esterase and pancreatic lipase on the two substrates tested: viz., tributyrin and ethyl butyrate.

Sodium tauroglycocholate at a concentration of 3.7×10^{-3}M inhibited esterase completely and activated lipase when tributyrin was used as substrate. The inhibition of esterase with ethyl butyrate as substrate was only partial.

A method for the estimation of lipase in plasma has been described.

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INCREASE IN OXYGEN CONSUMPTION OF HUMAN CARDIAC MUSCLE INCUBATED WITH LANATOSIDE C

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INFORMATION concerning the mode of action of digitalis on the human myocardium has been difficult to obtain in the past. Recently, a source of fresh tissue has been provided by means of cardiac biopsy at the time of thoracotomy,[1] and the behavior of the muscle in vitro has been studied. This report is an account of the effect of one of the cardiac glycosides on oxygen consumption of slices of human cardiac muscle.

METHODS*

The auricular appendage was removed at the time of thoracotomy when a nonresectable neoplasm was encountered in 3 cases and when mitral commissurotomy was performed in 2 cases. The muscle was placed at once in a Petri dish resting on ice, and slices 0.5 mm. thick were cut with a Stadie-Riggs slicer. Respiration was determined by the direct method of Warburg with oxygen as the gas phase. Carbon dioxide was absorbed with 0.1 ml. of 30 per cent KOH in the center well, and the slices were immersed in Krebs-Henseleit physiological saline (2.5 meq. per liter Ca) and incubated at 37.5° C. In one instance alcohol and glycerine in the same concentrations used to dissolve certain samples of lanatoside C were used in the main chamber. In later experiments the crystalline drug† was used. NaCl (0.9 per cent) was used as the medium with and without the glycoside in 2 determinations. Substrate and lanatoside C were added from the side arms of the vessels to make a total volume with the saline of 2.0 ml., and oxygen consumption was computed on the basis of dry weight of the tissue. The concentrations of glycoside and substrates used are indicated in Table I. The average weight of tissue used in each vessel was 89.8 mg.

RESULTS

The effect of lanatoside C on respiration of human cardiac muscle observed in these experiments is summarized in Table I. The data are tabulated as the number of cubic millimeters of oxygen consumed per milligram of dry weight of tissue, and, except where it is specifically indicated, the medium used was Krebs-Henseleit physiological saline. Addition of the same amount of alcohol and glycerine in which certain samples of glycoside were dissolved resulted in no higher rate of respiration (9.7 cu. mm. of oxygen consumed per milligram of dry tissue in four hours) than when saline medium was present alone (11.1 cu. mm. in the same period). Examination of results did not reveal any difference between the oxygen uptake of tissue after lanatoside C in alcohol-glycerine solution was added and the uptake after addition of a powdered sample of lanatoside

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TABLE I. EFFECT OF LANATOSIDE C ON RESPIRATION OF HUMAN CARDIAC MUSCLE

DETERMINATIONS	MEDIUM	MINUTES							
		30	60	90	120	150	180	210	240
6	Krebs-Henseleit	1.5*	2.5	3.3	4.2	5.0	5.4	6.5	7.3
1	0.9% NaCl	1.8	3.1	4.5	5.6	6.9	8.4	9.7	11.1
1	8% alcohol 15% glycerine	1.9	3.2	4.6	5.8	6.7	7.8	8.9	9.7
3	0.02M glucose	3.0	4.7	5.9	7.4	8.7	10.7	12.0	15.9
2	0.02M Na pyruvate	4.1	7.1	9.4	12.2	14.0	16.3	18.4	22.8
4	0.5 mg. % Lanatoside C	2.7	5.1	9.0	12.3	15.5	17.9	20.1	21.9
1	0.5 mg. % Lanatoside C 0.9% NaCl	3.6	6.2	8.8	13.0	15.9	19.0	21.4	24.9
2	0.5 mg. % Lanatoside C 0.02M glucose	1.9	3.7	6.9	10.3	13.5	18.5	21.5	
2	0.5 mg. % Lanatoside C 0.02M Na pyruvate	1.9	4.5	7.6	10.6	13.4	16.1	18.4	20.2
9	0.5 mg. % Lanatoside C Summary	2.4	4.8	8.2	11.6	14.6	17.6	20.0	22.1

*Mean cubic millimeters of oxygen consumed per milligram of dry weight of tissue.

C dissolved in physiological saline. The effect of the drug on respiration of the slices therefore could not be attributed to the solvent.

The muscle respired at approximately triple the rate found in control studies when lanatoside C was added to the medium. For example, an average of 7.3 ± 1.4 cu. mm. of oxygen was consumed per milligram in four hours in 6 control determinations, and an average of 21.9 cu. mm. of oxygen was utilized in the same length of time when 0.5 mg. per cent of lanatoside C was present with 4 different samples of muscle. When 0.9 per cent NaCl was used as the medium 11.1 cu. mm. of oxygen was consumed in four hours without addition of glycoside, and 24.9 cu. mm. of oxygen was utilized when lanatoside C was added. Addition of substrate to the slices increased average oxygen consumption from 6.5 ± 1.3 cu. mm. without exogenous substrate to 12.0 cu. mm. with 0.02M glucose and 18.4 cu. mm. with 0.02M sodium pyruvate in 3.5 hours. With lanatoside C and glucose the average oxygen uptake of the slices was 21.5 cu. mm., and with lanatoside C and pyruvate it was 18.4 cu. mm. in the same length of time. The 9 determinations in which lanatoside C was added to the medium both with and without substrate were averaged. At the end of four hours the mean oxygen consumption increased from 2.4 ± 1.1 cu. mm. in 0.5 hour to 22.1 ± 7.8 cu. mm. in four hours.

DISCUSSION

Gottschalk[7] found oxygen uptake of cardiac muscle of the frog with strophanthin to be equal to the control value in only one of her determinations. In the remainder oxygen consumption of the muscle was depressed, particularly at higher concentrations of the glycoside. Wollenberger[19] quotes the work of a number of Freund's students who studied the effect of aglycones and glycosides on the respiration of mammalian and frog heart muscle. Buddeberg, Friebel, Frühauf, and Pötting reported inhibition of oxygen uptake, but Krüger and

Frye reported enhancement of respiratory metabolism with concentrations in the range of $1:10^6$. Salomon and Riesser[15] found that neither digitoxin nor strophanthin affected oxygen consumption of the isolated frog heart and of minced tissue from hearts of frogs, mice, and rabbits. Using minced cardiac muscle from guinea pigs and rats, Genuit and Haarmann[6] were unable to demonstrate any clear-cut effect of strophanthin on oxygen uptake of the tissue. Dubois, Doull, and Geiling[3] found that bufagin inhibited the rate of carbohydrate oxidation of cardiac slices from the guinea pig, rat, and mouse.

Rohde and Ogawa[14] found that oxygen uptake of the isolated cat heart was increased by perfusing with 0.04, 0.05, and 0.06 mg. per cent strophanthin in Ringer's solution. Strophanthin in concentration of $1:10^7$ was found to increase oxygen uptake of isolated frog ventricle by Eismayer and Quincke,[4] but higher concentrations of the drug leading to systolic arrest diminished oxygen consumption. David[2] reported that the oxygen uptake of frog auricle was increased initially, followed by a decrease, and that higher concentrations depressed the uptake. Respiration of ventricular muscle of the frog increased 93 per cent on addition of ouabain in concentration of 3×10^{-5} in Ringer's solution, and the time of action of the drug on oxygen utilization was found inversely proportional to concentration in experiments reported by Victor.[16] Levy, Libert, and Schwob, in a series of papers,[8-12] described an increase in oxygen utilization by slices of mammalian heart in the presence of glycosides. This effect lasted approximately one hour, and with higher concentrations of glycosides the oxygen consumption of the slices was depressed. Wollenberger[17] found that the oxygen uptake of slices of cardiac muscle from the guinea pig was increased by 2 to 3×10^{-7} M ouabain in the presence of glucose or lactate. At higher concentrations this was followed by a depression of oxygen consumption. When pyruvate was present the oxygen uptake was high initially and depressed by ouabain without preceding stimulation. He suggested that the increased respiration was due to the glycoside facilitating entry of exogenous substrate into the cell, and the later inhibition to be due to loss of diffusible catalysts. This investigator has more recently modified his views concerning the mechanism of action which he proposed at that time. Using C^{14}-labeled glucose and slices of dog myocardium he[18] found that the stimulation of respiration by ouabain was not accompanied by a greater utilization of glucose, although the rate of glucose oxidation was doubled. In studies by Finkelstein and Bodansky[5] the oxygen uptake of ventricular slices from the cat was increased to greatest extent by 1×10^{-6} M scillaroside, digitoxin, and ouabain, and the acceleration was dependent on the concentration of the drug. Although a response was obtained without glucose, the acceleration of respiration was increased by addition of glucose up to a concentration of 0.2 per cent. Studies on the isometrically contracting rat auricle by Ransom and Loomis[13] revealed that tension increased the R.Q. of the muscle and that the R.Q. was lower when ouabain was added except that concentrations of 4.5×10^{-7} M brought it back to control levels. When there was no tension ouabain caused a higher R.Q., both oxygen uptake and CO_2 production being increased, but this was not due to increased auricular rate of contraction.

A biphasic response to ouabain was found, depending on the concentration of drug used. Wollenberger[19] has discussed other aspects of the action of the cardiac glycosides on the heart in a recent review.

It is evident that a number of investigators have found that glycosides increase oxygen utilization of cardiac muscle when they are present in certain concentrations. There may be a subsequent depression of oxygen uptake, and the effect of the drug may be dependent on its concentration. Increase in oxygen uptake has not been demonstrated in most instances where minced tissue was used and occasionally in other preparations.

The results with human cardiac muscle indicate that 0.5 mg. per cent lanatoside C causes increased oxygen consumption of the tissue when added either to Krebs-Henseleit or 0.9 per cent NaCl medium. Since crystalline material was used in most of the determinations and the solvent of the liquid preparation caused no increase in respiration, the increment apparently is due to the glycoside itself.

The effect is not dependent on exogenous substrate, and no conclusive evidence that the rate of respiration is increased additionally by the presence of substrate was found, although in one heart the oxygen uptake of slices with glucose and lanatoside C was 11.7 cu. mm.; with lanatoside C it was 7.2 cu. mm.; with glucose it was 6.7 cu. mm.; and with saline it was 5.4 cu. mm. per milligram dry weight in 2.5 hours. Glucose increased oxygen consumption of the muscle to less extent than either lanatoside C alone or lanatoside C plus glucose, when all determinations were averaged in each category. The average values for uptake of oxygen by the tissue were within the same range when lanatoside C, pyruvate, or lanatoside C and pyruvate were added to the medium.

The amount of glucose utilized with and without lanatoside C was found in three determinations. The average amount utilized without the glycoside was 1.02 mg. per 100 mg. dry weight of tissue per hour, and with lanatoside C it was 1.22 mg. Therefore, an average increase in glucose utilization of 0.20 mg. per 100 mg. dry weight occurred on addition of lanatoside C. Pyruvate utilization was determined for one sample, and 0.55 mg. more pyruvate was found to be utilized per 100 mg. dry weight of the muscle without the glycoside than with it. This phase of the study is being continued, utilizing compounds labeled with C^{14}.

Although the number of determinations is restricted by the limited availability of human tissue, there seems to be little doubt that lanatoside C has caused increased oxygen consumption of slices of human cardiac muscle when each determination is considered with reference to control studies on slices from the same heart or when the data are considered as a whole (Table I). The mechanism of this action and its relation to contraction are not clear, but it seems permissible to conclude that a direct effect of this cardiac glycoside on the human myocardium has been demonstrated.

SUMMARY

Lanatoside C caused an increase in oxygen consumption of human cardiac muscle in vitro.

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OBSERVATIONS ON SOME OF THE TECHNICAL AND CLINICAL FACTORS INFLUENCING THE DETERMINATION OF UROPEPSIN EXCRETION IN MAN

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ALTHOUGH it had been known for a long time that the urine contained an enzyme possessing proteolytic properties,[1] the application of this knowledge in clinical investigation has been quite recent. Mirsky[2] and his associates,[3, 4] as well as Bucher,[5] and Farnsworth and his co-workers[6] made outstanding contributions in their studies on the source and properties of uropepsin, as well as in the development of methods for uropepsin assay. Following the reports by Spiro, Reifenstein, and Gray[7] and by Gray, Benson, Reifenstein, and Spiro[8] that the administration of ACTH to normal individuals causes an increase in the secretion of gastric pepsin as well as in the excretion of uropepsin, it became apparent that this response might serve as a means of studying adrenal cortical function. Mirsky and associates[9] have studied changes in uropepsin excretion in patients with duodenal ulcer, and also in anxiety states, finding elevated uropepsin levels in a significant number of each of these conditions.

A simplified method for determining uropepsin excretion rates has recently been proposed by West and his co-workers.[10] In this method homogenized milk is used as the substrate for measuring uropeptic activity, and it is based upon the conversion of casein into paracasein. The end point of this reaction is subjectively noted by the appearance of protein precipitation on the side of the test tube during incubation of activated urine with buffered milk in a water bath at 37° C.

The purpose of this report is threefold, viz. (1) to present the results of our experience using the West uropepsin assay method, (2) to suggest a practical modification of that method, and (3) to comment generally on some of the pitfalls encountered in the process of evaluating uropepsin excretion rates.

MATERIALS AND METHODS

1. Control subjects consisted of normal healthy young adults selected at random from the clerical and research staff of a large hospital. Test subjects consisted of carefully studied patients who had been admitted to the hospital for a variety of gastrointestinal disorders. Uropepsin assays were performed in the manner described by West and co-workers[10] using a homogenized milk substrate. Following the notation suggested by West that one "unit" of uropepsin is the number of milliliters of activated urine times 10 required to give the end point in 100 seconds, urine samples obtained between 7:00 A.M. and 11:00 A.M. were tested.

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2. Different brands of homogenized milk, and milk products whose fat content varied widely, were tested as substrates using the West method.

3. A study of the diurnal variation in uropepsin output was made by testing urine samples collected four times a day for seven consecutive days.

4. The stability of the proteolytic activity of stored urine specimens was investigated. Each sample was divided into four equal portions and to two of these toluene was added. One pair (urine plus toluene; urine without toluene) was allowed to stand at room temperature, and the other corresponding pair was placed in a refrigerator. Their uropeptic activity was determined on the first, fourth, and ninth days.

5. A search for a substrate more uniform than homogenized milk was made. The details of its preparation and use appear under "results."

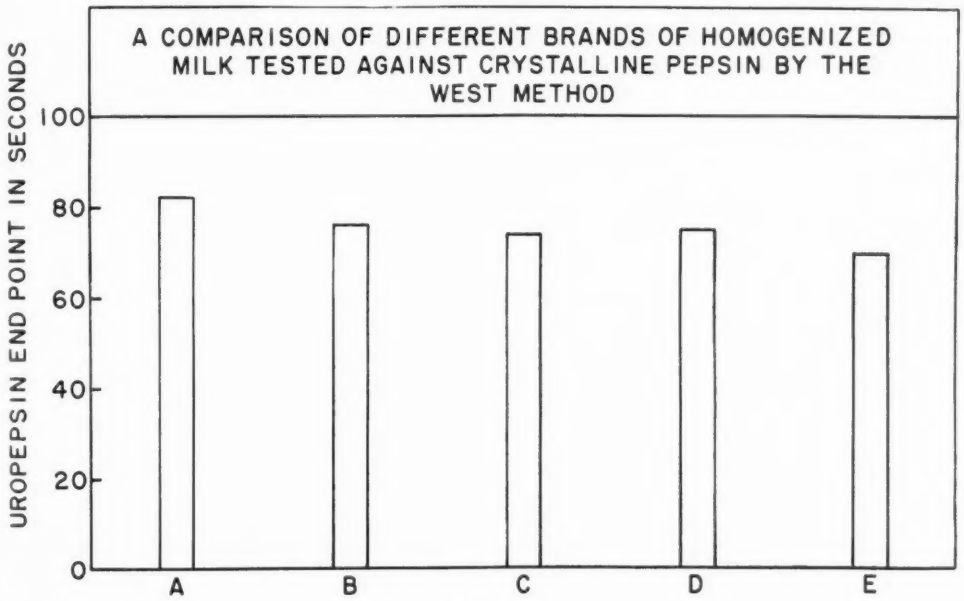

Fig. 1.

RESULTS

More than one thousand uropepsin assays have been made in this laboratory using a homogenized milk substrate in the manner described by West and co-workers.[10]

Fifty-nine determinations in 27 control subjects yielded a mean value of 28 units of uropepsin per hour; S.D. = 14.5. This checks closely with West's data which showed the normal range to lie between 15 to 40 units per hour.

Seventy-nine determinations in 30 subjects having active duodenal ulcers yielded a mean of 50 units per hour; S.D. = 42.0. Statistical analysis reveals that when the uropepsin activity is used as the sole basis for differentiating between the normal individual and one having an active duodenal ulcer, one is unable to make this choice in at least 35 per cent of the cases.

Patients with benign gastric ulcer and carcinoma of the stomach have uropepsin values falling within the normal range.

Patients with pernicious anemia and those who have been subjected to total gastrectomy show no uropepsin activity. One patient suspected of having pernicious anemia based upon hematologic findings was found to have a normal uropepsin output, and subsequently this individual was shown to have an aerestic anemia.

Three patients with chronic ulcerative colitis were given large doses of ACTH, and only one of them revealed an increase in uropepsin output. We have not tested the effect of giving ACTH to normal subjects.

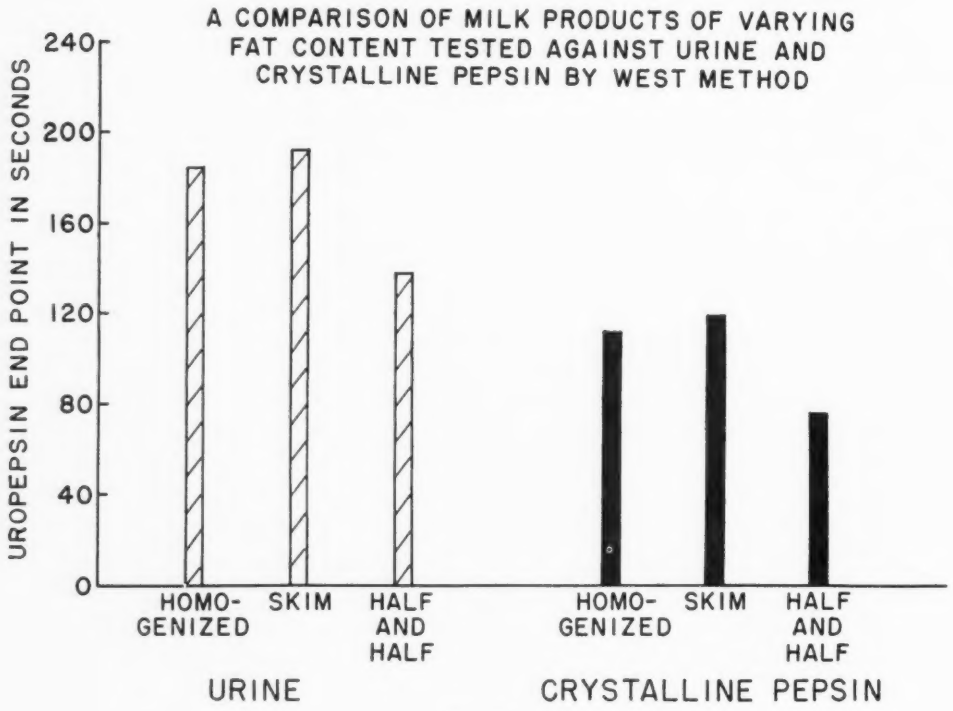

Fig. 2.

Fig. 1 shows a comparison of five different brands of homogenized milk (selected at random within the Los Angeles area) tested against a standard solution of crystalline pepsin, using the West procedure. Fig. 2 illustrates the marked differences obtained by testing both urine and pepsin on substrates of homogenized milk, skimmed milk, and half and half.

Fig. 3 clearly reflects the variations occurring in one of our subjects who was tested on seven consecutive days. This wide dispersion of results was typical of several other patients similarly studied. Fig. 4 shows that refrigeration without the addition of toluene results in the least change in uropeptic activity of urine left standing for several days. It is also apparent that the higher the initial activity of the urine, the greater will be the rate at which this activity is lost.

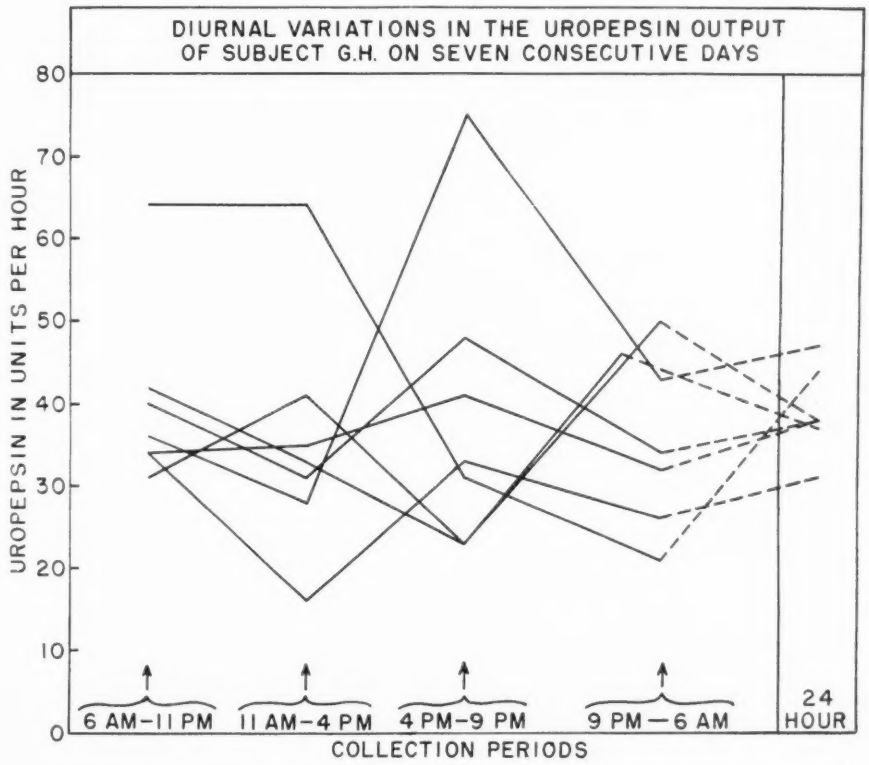
DIURNAL VARIATIONS IN THE UROPEPSIN OUTPUT
OF SUBJECT G.H. ON SEVEN CONSECUTIVE DAYS
UROPEPSIN IN UNITS PER HOUR
80
70
60
50
40
30
20
10
0
6 AM–11 PM
11 AM–4 PM
4 PM–9 PM
9 PM—6 AM
24 HOUR
COLLECTION PERIODS

Fig. 3.

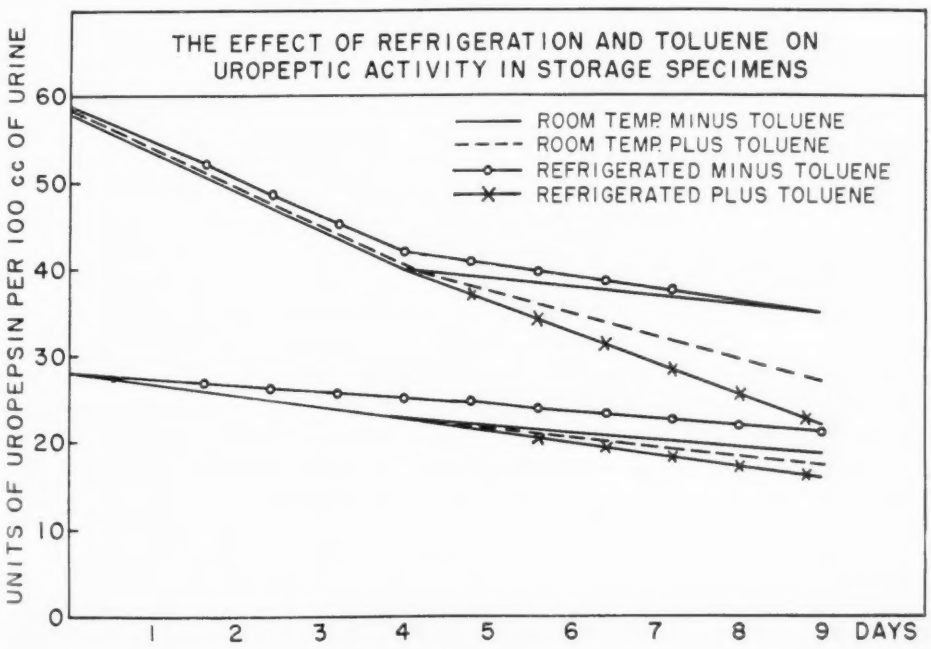
THE EFFECT OF REFRIGERATION AND TOLUENE ON
UROPEPTIC ACTIVITY IN STORAGE SPECIMENS
ROOM TEMP. MINUS TOLUENE
ROOM TEMP. PLUS TOLUENE
REFRIGERATED MINUS TOLUENE
REFRIGERATED PLUS TOLUENE
UNITS OF UROPEPSIN PER 100 cc OF URINE
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9 DAYS

Fig. 4.

We have devised a casein substrate and standardized our calibration so that assay end points can be directly calculated in terms of micrograms of pepsin. Casein obtained from two different manufacturers has yielded identical calibrations and the prepared substrates have remained stable for more than 14 days when stored in a refrigerator. The procedure for preparing this casein substrate appears below and the standardization graph is shown in Fig. 5.

Preparation of casein substrate for West uropepsin assay reagents:

(1) 1.0N sodium hydroxide
(2) 1.0N acetic acid
(3) Acetate buffer of pH 4.9 (NaOH 4.2 Gm.,
glacial acetic acid 9.2 ml.,
distilled water to make 100 ml.
(4) Calcium chloride
(5) Acid free casein (obtained from General
Biochemicals, Inc., Chagrin Falls, Ohio,
and containing):

a. Protein	89.0 per cent
b. Fat	0.5 per cent
c. Lactose	0.0 per cent
d. Lactic acid	0.1 per cent

Preparation of Substrate.—Into a 100 ml. volumetric flask place 0.3 Gm. of acid-free casein. Add 20 ml. of distilled water and exactly 10 ml. of 1.0N NaOH. The flask should be rotated gently while the sodium hydroxide solution is added slowly. After the casein has completely dissolved, slowly add 12 ml. of 1.0N acetic acid and continue to gently rotate the flask to ensure adequate mixing without producing bubbles. To this mixture add 10 ml. of a solution containing 1.2 mg. of calcium chloride in acetate buffer of pH 4.9. Fill the volumetric flask to the 100 ml. mark by adding only acetate buffer of pH 4.9.

Assay.—The details of the assay are performed exactly as stated by West and collaborators, except that 0.5 ml. of this casein preparation is used in place of 0.5 ml. of homogenized milk. The end point consists of a slight aggregation of casein particles appearing along the sides of the test tube just above the surface of the solution. To facilitate detection a black painted copper square is placed on the bottom of the water bath and the test tube is shaken gently every ten to fifteen seconds until the precipitation appears in the fluid film on the walls of the tube. Determinations are most reliable when the end points fall between fifty and two hundred seconds. Fig. 5 illustrates how easily timed end points are related to uropepsin activity as based upon the corresponding activity of crystalline pepsin in micrograms per milliliter.

DISCUSSION

In our experience the West uropepsin assay is generally a very satisfactory procedure. From a technical viewpoint this test is simple, rapid, and inex-

pensive. Duplicate runs yield consistently similar results. However, in evaluating the general usefulness of any enzyme assay method it is apparent that a given substrate should be as uniform as possible. It did not seem likely, for example, that homogenized milk in Los Angeles should necessarily be identical to homogenized milk in Chicago, New York, or Boston. Moreover, it was probable that different brands of homogenized milk within the same locality might differ considerably. The results reported in this paper indicate that significant differences do exist. The reasons for these observed differences are not known. One explanation might be that detection of the end

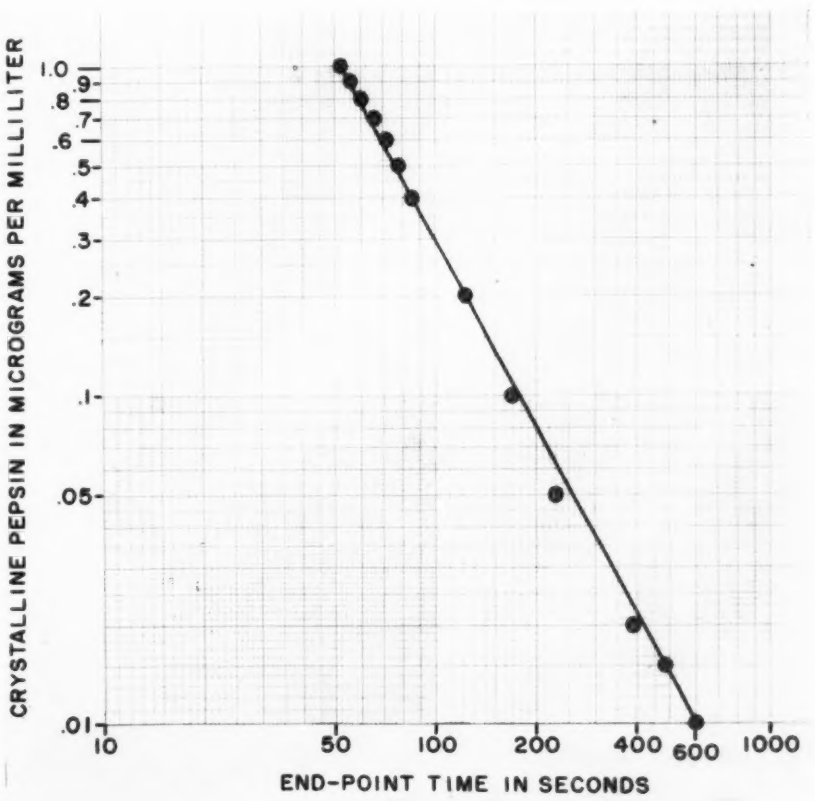

Fig. 5

point is related to the fat content of the substrate, since the fat particles provide a surface upon which the protein particles distribute themselves and hence facilitate the detection of protein precipitation. These data suggested the need for a more uniform substrate.

Many authors still prefer to define their own "units" rather than employ a more basic reference when such is available. Since uropepsin assays are based upon a comparison with crystalline pepsin it certainly seems reasonable to express results in terms of micrograms of pepsin or uropepsin.

Regarding the collection of timed urine samples, our experience does not confirm the statement of West and co-workers[10] that the morning excretion rate of uropepsin in healthy individuals shows little variation in the same subject from day to day. We are in agreement with those who have advocated testing an aliquot portion of a twenty-four hour urine collection. Furthermore, we advise that testing be done within twenty-four hours after completing the urine collection and that interim collections prior to testing be kept in a refrigerator without adding toluene.

It has previously been reported[5] that diets low in protein are accompanied by a decrease in uropepsin output and, correspondingly, that high protein intakes are associated with an elevated uropepsin output. In one carefully studied subject whose uropepsin outputs were normal on a 2,800 calorie diet containing approximately 70 Gm. of protein, no significant changes in uropepsin output were noted after increasing the protein to 150 Gm. daily for seven consecutive days. Extremely low protein intakes were not studied. We have also observed that drastic changes in fluid intake have not been accompanied by any significant alterations in uropepsin excretion.

We have not had a sufficiently wide experience in studying uropepsin excretion to reach any conclusion as to its ultimate usefulness as a clinical or investigative aid. Although it has been suggested[8, 10] that uropepsin excretion is a reflection of adrenal cortical activity, we feel that considerably more data must be accumulated before uropepsin excretion may be used as a measure of adrenal cortical function. The level of uropepsin excretion is of no practical value in distinguishing a person having duodenal ulcer from a normal healthy individual. In the presence of normal renal function it appears to be an accurate qualitative index of gastric mucosal function and, as such, may serve to confirm the diagnosis of pernicious anemia or the adequacy of total gastrectomy.

SUMMARY

A new method for measuring uropepsin whereby homogenized milk is used as the substrate has recently been proposed. Our experience with over 1,000 tests by this method is reported. Some of the technical and clinical pitfalls of this test have been discussed.

A modification of the method is presented whereby casein, instead of homogenized milk, is employed as the substrate. The casein substrate has the advantage of being stable, inexpensive, uniform, and generally available. The basic principles involved in the West uropepsin test have not been altered by this modification.

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DEXTRAN AND OXYPOLYGELATIN AS PLASMA VOLUME EXPANDERS: RENAL EXCRETION AND EFFECTS ON RENAL FUNCTION

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FORT SAM HOUSTON, TEXAS

WITH THE TECHNICAL ASSISTANCE OF CORPORAL IVAN T. STINSON, ARMY OF THE UNITED STATES

THE need for a suitable plasma volume expander that can be used when the supply of whole blood or plasma is insufficient has led to the production of several new colloid solutions. Two of the most promising materials are dextran,[9] a glucose polysaccharide developed in Sweden and now manufactured in this country as well, and oxypolygelatin (OPG),[4] a modified gelatin which remains fluid at low temperatures. Previous studies from this laboratory indicated that both are effective in the treatment of hemorrhage in experimental animals,[11, 15] but revealed some differences in their ability to expand blood volume. The present report deals with renal excretion and the effects on renal function of these two expanders. The kidney is the chief avenue of early loss of these materials. This loss is probably due to filtration at the glomerulus, and its rate is affected by molecular size. Renal excretion studies may thus give an index of the ability of dextran and OPG to remain within the vascular tree.

Since restoration and maintenance of renal function is an important aim in the treatment of shock, studies of any adverse effects on the kidney are important in the evaluation of a plasma volume expander. Michie[13] and Froeb and associates[6] have reported depressions of maximum tubular excretion of para-aminohippurate (Tm_{PAH}) after administration of dextran, but found no other changes in renal function; OPG has not previously been studied but Michie and others[14] have found no adverse effects from the administration of gelatin.

We have compared the rate of excretion of dextran and OPG with their rate of disappearance from the blood in dogs with a normal blood volume (expansion studies) and dogs subjected to standard hemorrhage (replacement studies). The effects of single and multiple daily infusions on renal hemodynamics and salt and water excretion were studied in normal animals. Similar data for animals subjected to a standard hemorrhage were previously reported.[11]

MATERIALS

Dextran.—Six per cent dextran in isotonic saline (Expandex—Commercial Solvents Corporation, Lot Nos. 84647, 84681, 84684, 84695, and 81907) was used. Not all of these

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lots have been characterized, but other material in the 846 series was found to have a number-average molecular weight of about 40,000, with a range of 31,000 to 56,000.[1]

Oxypolygelatin.—Five per cent OPG (Don Baxter, Inc., Lot Nos. 8141X and 12676AY) in isotonic saline was used. Lot 8141X, which was used in most experiments, is said by the manufacturer to have a number-average molecular weight of 20,000; while 12676AY, an early preparation, has a weight of about 15,000.

Infusions of 4.5 per cent human albumin in isotonic saline and isotonic saline alone were used in control experiments.

METHODS

Replacement Studies.—Dextran and OPG were given as volume replacement after massive hemorrhage in anesthetized dogs. The technique of bleeding and replacement has been reported previously.[11] This hemorrhage and infusion averaged about 50 ml. per kilogram of body weight. Renal excretion under these conditions was studied in eight dogs.

Expansion Studies.—Five normal female dogs were used. All infusions amounted to 20 ml. per kilogram of original body weight. Eight single infusion experiments were performed in two trained unanesthetized dogs; in these animals dextran, OPG, saline, and 4.5 per cent human albumin in isotonic saline were given. Seven multiple infusion experiments were performed in five dogs. In Experiment 1, three infusions were given in ten days, and in Experiment 2, eleven infusions were given in fourteen days, while in the remainder of the experiments infusions were given daily. Experiment 7 was a continuation of Experiment 6. Excretion and clearance studies were conducted with the first and last infusion of each experiment. Tm_{PAH} was determined from the average of three or more clearance periods obtained before the first infusion and after the last infusion in five experiments. Nembutal anesthesia (32 mg. per kilogram) was used in five of these experiments.

The animals were fasted but had free access to water for eighteen hours prior to each clearance study. In the single infusion experiments, 500 ml. of isotonic saline were given orally one and one-half hours prior to infusion. After a suitable priming dose of creatinine and para-aminohippurate, a sustaining infusion containing these substances in isotonic saline was given at a rate of 2 ml. per minute. After two or more fifteen- to thirty-minute control periods, the test infusion was given rapidly (in four to twelve minutes) and observation continued for three to five hours, usually with half hour urine collection periods.

Glomerular filtration rate (GFR) was measured with creatinine; and effective renal plasma flow (ERPF) with low concentrations of para-aminohippurate as described previously.[11] Tm_{PAH} was determined with suitably high concentrations of para-aminohippurate.[7] Sodium and potassium were measured in a Perkin-Elmer flame photometer.

Plasma volume was measured before and after infusion by repeated injection of the blue dye, T-1824, as described previously.[15] Plasma protein was measured with the Biuret reagent.[8] Hematocrit was measured in Wintrobe tubes centrifuged for twenty-five minutes at 2,500 r.p.m.

For the calculation of blood retention of expander for periods between T-1824 plasma volume determintations, a constant red cell volume was assumed and plasma volume calculated from hematocrit changes. These derived volumes were multiplied by plasma concentration for the calculation of blood retention; where previous infusions had been given, the amount of circulating expander prior to infusion was subtracted from the total determined after infusion.

Dextran concentration in plasma and urine was determined by Bloom's method.[3] OPG in plasma and urine was measured by Siiteri's method.[16]

RESULTS

I. Renal Excretion.—The urinary excretion of dextran and OPG was rapid and uniform in the 12 studies of the expansion series (Fig. 1). About 25 per

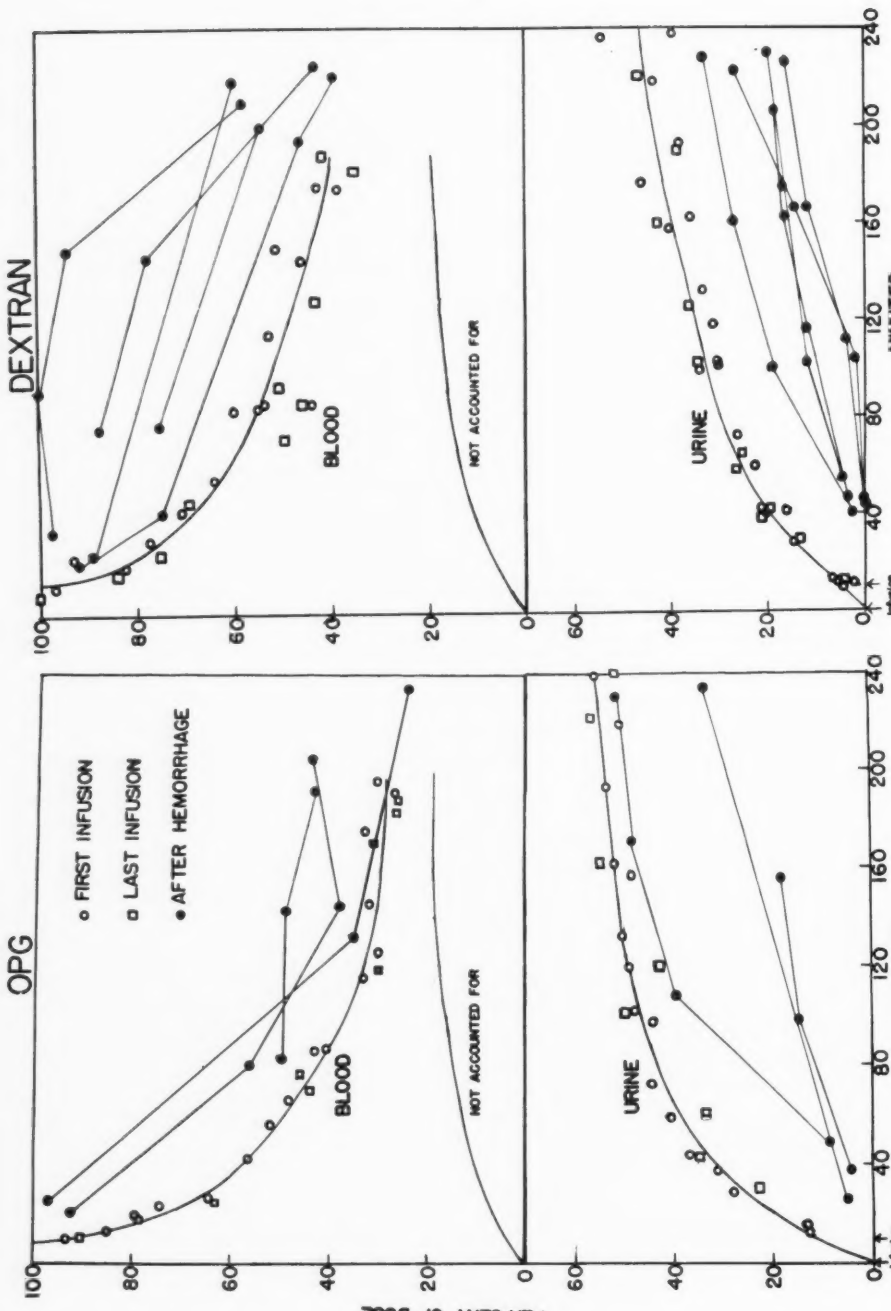

Fig. 1.—Urinary excretion and blood retention of OPG and dextran. The amount not accounted for in the blood and urine is given as an average for the expansion series.

cent of the infused dextran was excreted in one hour and 50 to 60 per cent in four hours. About 40 per cent of infused OPG (Lot No. S141X) was excreted at one hour and 50 to 60 per cent at four hours. The earlier OPG preparation (Lot No. 12676AY) of lower molecular weight was given in three experiments (not shown in Fig. 1) and was excreted more rapidly; about 55 per cent was recovered in the urine in one hour and 75 per cent in three hours. Both expanders were lost from the blood more rapidly than they appeared in the urine.

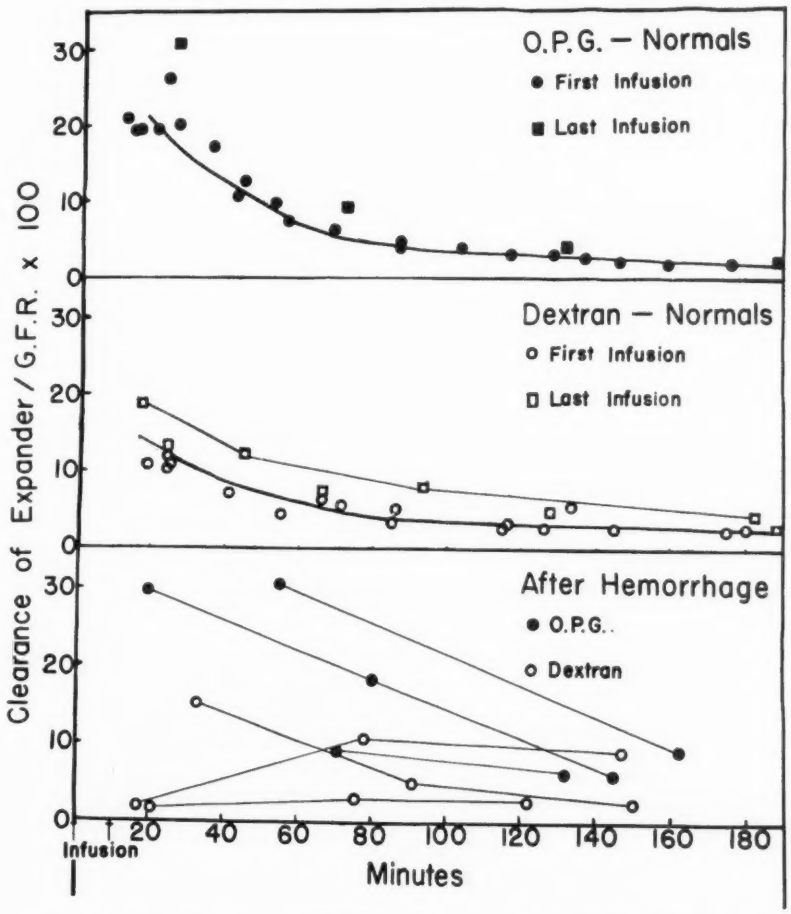

Fig. 2.—The clearance of dextran and OPG. The connected squares in the "Dextran-normals" series represent an experiment (Experiment 7, Table II) in which expanders clearance was the same as in the first infusion, but filtration rate diminished.

At three hours only 35 to 45 per cent of infused dextran and 25 to 35 per cent of OPG were retained in the blood. Thus there is a portion of the infused expander unaccounted for in plasma and urine which gradually increases to about 20 per cent at three hours. As can be seen from the values obtained for the last infusion (Fig. 1), multiple infusions did not alter excretion or blood retention appreciably.

After hemorrhage and replacement greater variation in excretion and retention was expected, since renal function and plasma volume also varied considerably.[11] As can be seen in Fig. 1, dextran excretion was decreased to as little as 15 per cent and OPG excretion to 30 per cent in four hours.

The renal clearance of both dextran and OPG was not constant but decreased with time; i.e., the rate of excretion of expander was not directly proportional to plasma concentration. When the clearance was expressed as per cent of GFR (Fig. 2) differences in animal size were factored out and consistent values were obtained from dog to dog. Twenty minutes after infusion OPG clearance was 20 to 30 per cent and dextran clearance 10 per cent of GFR. Both declined to about 2 per cent at three hours. With lower molecular weight OPG (Lot No. 12676AY), clearance was 30 to 40 per cent of GFR twenty minutes after the infusion and fell to 5 per cent by three hours. Since the first expander clearance is calculated from a thirty- to forty-minute postinfusion urine collection period, it is probably not a maximal value. Expander clearance was not altered by multiple infusions, but the clearance ratio was increased in one dextran experiment by virtue of a fall in filtration rate.

In animals subjected to hemorrhage expander clearance usually bore a similar relationship to GFR. However, in the first postinfusion period in two animals given dextran, expander clearance was depressed to 2 per cent of GFR, and urinary excretion was correspondingly low.

While retention of the expander itself was uniform from dog to dog in the expansion series, there was marked variation in fluid retention among the different groups of dogs studied (Table I). One-fourth hour after the first infusion of a series, plasma volume expansion was approximately equal to the amount of fluid administered. Plasma volume then rapidly declined to preinfusion values or lower. After the last of a series of multiple infusions or after saline loading, plasma volume increased by 130 to 190 per cent of the amount of fluid given, and at three hours the plasma volume was still increased by 30 to 90 per cent of the volume infused.

TABLE I. CHANGES IN PLASMA VOLUME FOLLOWING EXPANDER INFUSION

		DOG (NO.)	DOSE OF EXPANDER (C.C.)	PLASMA VOLUME PREINFUSION (C.C.)	△ PLASMA VOLUME POST-INFUSION ¼ HR. (C.C.)	3 HR. (C.C.)
First infusion	Dextran	9	320	445	+315	+ 40
		114	210	758	+208	- 67
	OPG	119	214	770	+197	-134
Last infusion	Dextran	9	320	672	+608	+291
		114	210	716	+350	+ 84
	OPG	119	214	703	+515	+ 76
Oral saline load	Dextran	9	320	769	+488	+301
		119	255	984	+448	+120
	OPG	9	320	881	+428*	+301
		119	255	951	+324	+ 92

*Plasma volume calculated from hematocrit change. All other determinations with T-1824.

TABLE II. SODIUM, POTASSIUM, AND WATER EXCRETION, AND RENAL HEMODYNAMIC RESPONSE AFTER DEXTRAN, OPG, SALINE, AND HUMAN ALBUMIN INFUSIONS*

		DEXTRAN					OPG					SALINE					ALBUMIN				
DOG. NO.	PERIOD	NA (μEQ./MIN.)	K (μEQ./MIN.)	V (C.C./MIN.)	GFR (C.C./MIN.)	ERPF (C.C./MIN.)	NA (μEQ./MIN.)	K (μEQ./MIN.)	V (C.C./MIN.)	GFR (C.C./MIN.)	ERPF (C.C./MIN.)	NA (μEQ./MIN.)	K (μEQ./MIN.)	V (C.C./MIN.)	GFR (C.C./MIN.)	ERPF (C.C./MIN.)	NA (μEQ./MIN.)	K (μEQ./MIN.)	V (C.C./MIN.)	GFR (C.C./MIN.)	ERPF (C.C./MIN.)
9	Control	363	51	4.4	60	159	345	67	3.1	57	160	531	71	5.2	61	164	728	87	4.8	61	148
	1	918	79	7.8	67	256	910	73	5.7	72	213	1015	68	6.6	70	211	1446	142	10.2	69	241
	2	769	52	4.8	68	220	603	31	3.1	74	218	703	55	4.4	69	214	952	56	5.4	70	211
	3	545	73	3.3	68	240	498	42	3.1	67	230	740	72	4.6	75	251	759	47	4.0	71	245
	4	479	58	2.7	68	220	362	35	2.1	68	253	730	71	4.2	77	277	681	48	3.7	75	295
	5	334	40	1.9	70	186	366	43	2.1	76	240	478	67	2.6	69	219	508	45	2.9	73	235
	6	324	42	2.0	77	262	360	50	2.2	76	215	382	58	2.1	74	194	410	38	2.2	72	210
	Post-infusion Average	562	57	3.8	70	231	517	46	3.1	72	228	675	65	4.1	72	223	793	63	4.7	72	240
119	Control	543	23	3.2	62	144	388	13	2.2	57	129	480	28	3.5	56	131	466	31	3.0	60	194
	1	861	49	5.3	83	216	1130	47	7.1	74	224	840	31	5.5	63	142	848	66	6.0	69	262
	2	1107	66	11.2	86	311	544	17	3.7	72	185	1080	53	9.5	73	206	875	46	8.7	77	356
	3	853	69	7.6	84	301	569	17	3.6	69	202	1054	--	9.2	72	216	882	77	8.7	74	435
	4	651	56	5.4	80	242	953	37	6.4	76	245	839	55	6.8	71	191	708	74	6.6	73	504
	5	433	57	5.3	80	212	875	41	6.0	80	237	440	--	4.3	66	165	365	51	3.4	68	362
	6	389	56	3.3	78	196	494	22	2.7	83	196	346	34	3.2	58	151	220	38	2.2	65	357
	Post-infusion Average	713	59	6.4	82	263	761	30	4.9	76	215	767	43	6.4	67	179	650	59	5.9	71	379

*Five hundred millimeters of oral saline was given one and one-half hours prior to infusion. Six thirty-minute urine collection periods were taken after infusion.

TABLE III. MULTIPLE

	EXPERIMENT NO.	DOG NO.	NO. OF INFUSIONS	VOL. OF EACH INFUSION (C.C.)	WEIGHT NGM.		PREINFUSION PLASMA VOL. (C.C.)		BLOOD VOL. (C.C.)		HEMATOCRIT (PER CENT)	
					a	p	a	p	a	p	a	p
OPG	1	9	3*	300	15.6	17	---	---	----	----	42	44
	2	63	11†	200	10.0	9.2	526	546	863	881	39	38
	3	119	12	214	10.7	10.5	770	703	1149	1192	33	33
	4	167	15	235	11.6	11.8	768	713	1164	1132	34	37
Dextran	5	9	6	320	16.3	15.6	445	672	857	1139	48	42
	6	114	6	210	10	11	758	895	1194	1398	37	36
	7	114	19‡	210	10	9	758	696	1196	967	37	29

Measurements were made before the first and before the last infusion, except for T_{mPAH} which was measured before the first and after the last infusion, and the renal hemodynamic response which was averaged for the three hours immediately following the first and last infusions.

*Three infusions given in ten days.

†Eleven infusions given in fourteen days. Lot No. 12676AY used.

‡Continuation of previous series.

II. Effects on Renal Function Following a single infusion of dextran, OPG, human albumin, or saline alone in unanesthetized dogs, prehydrated with oral saline, there is a rise in GFR and ERPF and in the excretion of sodium, potassium, and water (Table II). Filtration rate increases were usually well sustained during the three hours of observation, and were similar for all four substances. Renal plasma flow (and renal blood flow) reached a definite peak during the first two hours after infusion and subsequently declined. Albumin caused a greater increase in ERPF than dextran and OPG, while saline changed ERPF the least. The greater effect of albumin did not depend on plasma volume expansion since it caused less increase in plasma volume than dextran or OPG.

Sodium excretion reached a peak in the first or second half hour after infusion and then gradually declined toward preinfusion values. The average postinfusion sodium excretion was similar for all four materials in dog No. 119. The response was more variable in dog No. 9.

III. Multiple Infusion Experiments.—The effects of multiple infusions of dextran and OPG are summarized in Table III. Infusions of OPG did not cause any persistent changes in weight, plasma, or blood volume, hematocrit, GFR, or ERPF. T_{mPAH} was unchanged in one dog, while in the other (Experiment 4) it fell from a control value of 7.4 to 4.8 mg. per minute. This change was transient; the value rose to 6.3 nine days after the last infusion, and to 8.4 mg. per minute two weeks later. The twenty-four hour postinfusion plasma concentration of OPG rose slowly with repeated infusions and then usually reached a plateau. In Experiment 4, this concentration remained between 0.6 and 0.7 Gm. per cent from the eighth to the fifteenth infusion. Despite this plasma concentration, the urine contained only 0.05 Gm. per cent OPG just before the last infusion yielding a renal clearance of only 0.1 ml. per minute. In this experiment plasma protein concentration decreased to approximately the extent that

INFUSION EXPERIMENTS

VALUES									RENAL HEMODYNAMIC RESPONSE			
PLASMA CONCENTRATION OF EXPANDER (GM.%)	PLASMA PROTEIN (GM.%)		GFR (C.C./MIN.)		ERPF (C.C./MIN.)		Tm_{PAH} (MG./MIN.)		AVERAGE POSTINFUSION VALUES AS % OF CONTROL			
									GFR		ERPF	
p	a	p	a	p	a	p	a	p	a	p	a	p
0.1	- -	- -	58	55	150	147	- -	- -	117	115	128	128
0.2	- -	- -	49	47	---	---	4.9	5.0	108	101	---	---
0.3	5.8	6.2	28	28	94	89	- -	- -	112	109	136	132
0.6	4.7	4.0	40	43	93	---	7.4	4.8	---	---	---	---
1.4	5.5	4.9	58	55	168	203	8.9	7.8	129	110	150	113
0.7	4.6	4.1	41	36	88	---	3.4	4.1	---	---	---	---
1.2	4.6	4.1	41	27	88	102	3.4	3.7	97	119	152	114

OPG was retained. In Experiment 2, there was less retention of OPG and plasma protein concentration did not decrease. In the OPG experiments there was slightly less increase in GFR in response to the last infusion than to the first, but the ERPF response was the same.

Dextran showed evidence of greater retention than did OPG. After six infusions the plasma concentration was 1.4 Gm. per cent in Experiment 5 and 0.7 Gm. per cent in Experiment 6. The plasma protein concentration had decreased 0.6 and 0.5 Gm. per cent, respectively. In Experiment 5 the urinary concentration of dextran was 0.03 Gm. per cent and the renal clearance was 0.05 ml. per minute just before the last infusion. Plasma and blood volume was also increased while hematocrit fell. When further infusions were given to dog No. 114 (Experiment 7) the twenty-four hour postinfusion plasma dextran concentration reached 1.1 Gm. per cent after 11 infusions and remained near that value until the last infusion. However, plasma and blood volume fell to below control levels. In addition, there was a decrease in GFR, and the animal lost weight. At autopsy one week after the last infusion this animal was found to have a chronic interstitial nephritis which may have played a role in the changes in Experiment 7.

In Experiment 5 Tm_{PAH} fell slightly after the last infusion. In Experiment 7 Tm values were not decreased, in spite of a fall in GFR. An increase in ERPF was observed before the last infusion in Experiments 5 and 7. This change could account for the smaller per cent response of ERPF to the last infusion.

DISCUSSION

The rapid urinary excretion of dextran and OPG which we have observed in normal dogs has also been observed in man. Siiteri and Gray[16] have found the five-hour excretion of OPG to be 55 to 70 per cent of the dose when 500 ml. are given to normal man. Higgins and associates,[12] reported approximately 50 per cent excretion during the same time interval. Bloom[2] found that 33 per cent of dextran was excreted in six hours after an infusion of 500 ml. in normal men. A somewhat more rapid excretion is expected in dogs, since their glomerular filtration rate is higher in proportion to body size. The fall in renal clearance

with time is also to be expected. Both expanders contain a wide range of molecular weights. The smaller molecules are cleared rapidly, and the retained material has a higher average molecular weight and, therefore, a decreased renal clearance. The alternate possibility that a constant tubular reabsorption accounts for this change seems to be ruled out by the multiple infusion experiments. After the first infusion the usual plasma concentration was about 1 Gm. per cent, while renal clearance was 10 to 20 ml. per minute; after repeated infusions twenty-four hour plasma concentrations of 0.6 to 1.4 Gm. per cent were attained with a clearance of 0.1 c.c. per minute or less.

The gradually increasing fraction which is not accounted for in urine or blood probably represents material in the interstitial fluid. In his recent studies of this phenomenon Gropper[10] has demonstrated the presence of dextran in lymph.

In shock two variables may cause marked variations in excretion and retention of expanders. Diminished renal function would, of course, affect urinary excretion. Changes in capillary permeability could also affect the movement of these materials into interstitial fluid. In two dogs given dextran after hemorrhage, dextran excretion remained depressed after glomerular filtration had increased indicating either diminished glomerular permeability to this material or tubular reabsorption of large amounts of material.

Although no specific attempt has been made to demonstrate the inverse relationship between molecular size and excretion in this study, the more rapid excretion of OPG than dextran, and, more particularly, the very rapid excretion of the lower molecular weight OPG preparation (12676AY), bear out the probability of this relationship.

The wide variations observed in fluid retention, despite uniform loss of the material itself, indicate the extent to which plasma volume can be adjusted by normal dogs. Gropper[10] has found that in both hemorrhaged and nonhemorrhaged dogs, fluid and protein shifts between blood and other body compartments occur erratically and are independent of the amount of circulating colloid. In our experiments the escape of fluid from the plasma was slower after multiple infusions of expander or after preloading with oral saline than after the first infusion in dogs not previously hydrated. Under these circumstances it appears that plasma volume expansion is better maintained if there is concurrent expansion of the whole extracellular fluid volume.

In dogs there is little difference between the renal response to expanders and the response to a saline infusion. Renal plasma flow probably responds to plasma volume expansion but even here human albumin caused a greater increase in ERPF and less increase in plasma volume than did the expanders. Albumin may have some special effect on renal hemodynamics, since it also causes greater increases in human beings, compared to dextran[5] and gelatin.[14] The increase in sodium and water excretion after all infusion materials could be explained by their sodium and water content. There is no evidence for a specific diuretic effect of OPG or dextran.

Michie[13] has suggested that dextran may be nephrotoxic, since he observed depression of Tm_{PAH} in human beings three days after a series of six dextran

infusions at a time when dextran excretion was not detectable. Froeb and associates[6] observed depression in Tm_{PAH} after dextran was used to treat hemorrhagic hypotension in dogs. However, the decrease persisted only to the fifth or ninth day posthypotension. They also found histologic evidence of dextran deposition in the renal tubules twelve days after infusion in these dogs. In our small series dextran did not depress Tm_{PAH} significantly. A transient fall was observed following one series of OPG infusions with a return to normal values in three weeks. It may be that dextran deposition in the tubules is the cause of the Tm changes noted by Michie. If the gradual removal of this material results in a return to normal values, then serious nephrotoxicity seems unlikely. Evaluation of this concept in man is in progress.

The difference noted in the retention of multiple infusions of dextran and OPG probably is due to the greater number of large molecules (which cannot be excreted by the kidney) in the former preparation.

The small molecules of OPG and dextran are not effective as plasma volume expanders and indeed may be undesirable. They may cause a transient initial shift of fluid into the plasma during infusion and they may be excreted in high concentration (as high as 13 Gm. per cent was measured in the urine of one of our animals after hemorrhage). It would be desirable to narrow the molecular weight range of both materials by removing the smaller molecules.

SUMMARY

Dextran and oxypolygelatin (OPG) are rapidly excreted by the dog. When infusions are given to dogs with a normal blood volume 50 to 60 per cent of OPG and 40 to 50 per cent of dextran can be recovered in the urine in four hours. Part of the remainder is retained in the blood but about 20 per cent cannot be accounted for and has presumably entered interstitial fluids.

When the expanders are given after hemorrhage, excretion is slower and more variable, particularly with dextran.

The clearance of dextran and OPG decrease with time. During the first half hour after infusion dextran clearance was 10 to 15 per cent of glomerular filtration rate, while OPG clearance was 20 to 30 per cent. Both decreased to about 2 per cent of filtration rate in three hours.

Although the retention of the material itself is quite uniform in the expansion series, plasma volume changes are highly variable and appear to depend on the state of hydration of the animal.

The renal response to infusions of dextran and OPG is not consistently different from the response to the same volume of isotonic saline solution except that there is a greater increase in renal plasma flow with the colloid solutions.

Multiple infusions of dextran and OPG have minimal deleterious effects.

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MENINGOCOCCIC MENINGITIS: TREATMENT WITH LARGE DOSES OF PENICILLIN COMPARED TO TREATMENT WITH GANTRISIN

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RESULTS of treatment of meningococcic meningitis with sulfonamides have been so successful that therapy with these agents is considered to be the method of choice by most investigators. Soon after penicillin came into use, it was given a limited trial in meningococcic infections. The results were not as good as those that have been obtained with sulfonamides, even when penicillin was administered by the intrathecal as well as the intramuscular routes.[1-3] Dowling and his associates[4] have shown that when penicillin was administered intramuscularly in doses of 1,000,000 units of a soluble salt every two hours to patients with pneumococcic meningitis, better results were obtained than when penicillin was given in small parenteral doses plus intrathecal doses in addition to the sulfonamides.

The establishment of an additional reliable therapeutic regimen for a condition as serious as meningococcic meningitis seemed to us to be desirable, since the alternative regimen could be used for patients who were hypersensitive to sulfonamides and for those in whom the differential diagnosis between pneumococcic meningitis and meningococcic meningitis was uncertain.

For this reason we have treated alternate patients with meningococcic meningitis either with sulfonamides or with large parenteral doses of penicillin, and are reporting the results of this study in the present paper.

METHOD OF STUDY

Alternate patients who were diagnosed on admission to the hospital as having meningococcic meningitis were treated with Gantrisin or penicillin. No selection of patients was practiced, except that all patients who were considered on admission to have fulminating meningococcemia, with or without meningitis, were removed from the series and were given both penicillin and sulfonamides. This was done because the prognosis for such patients was so poor that we did not believe that we should withhold any agent which might have had possible therapeutic value.

The patients who received Gantrisin (Gantrisin group) were given 0.05 to 0.06 Gm. (¾ to 1 grain) per pound of body weight intravenously as an initial dose, and thereafter 0.1 to 0.13 Gm. (1½ to 2 grains) per pound for each twenty-four hour period, in divided doses, either by mouth or parenterally depending on the ability to retain oral medication.

In the penicillin group the dose was 1,000,000 units of the sodium or potassium salt intramuscularly or intravenously every two hours. If the patient was doing well at the

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end of twenty-four to forty-eight hours, 600,000 units of procaine penicillin in aqueous suspension was substituted for every third dose of penicillin for the next twenty-four hours, and if the course was still favorable, the soluble salt was discontinued and injections of the procaine penicillin suspension were continued at six-hour intervals. In infants the dose of procaine penicillin was decreased to 600,000 units every twelve hours on about the fifth day. Only in one patient in whom the change from a soluble to an insoluble salt of penicillin was made did there seem to be any difficulty from the change. In this instance the change was made twelve hours after the initial dose, an interval which we now consider to be too short. Patients with fulminating infections, besides being given Gantrisin and penicillin as described above, also received large doses of plasma, blood, adrenal cortical extract and, in later cases, cortisone.

During the course of the study we had an opportunity to study two other groups of patients: (1) those who had received two or more therapentic agents before admission to the hospital and who were therefore continued on combination therapy, and (2) those who were started on combination therapy because the diagnosis was in doubt when the patient was admitted to the hospital.

Patients in the first group had been given sulfonamides and moderately large doses of penicillin before admission and were continued on both drugs in the hospital in doses of approximately 1.2 to 2.4 million units of procaine penicillin in aqueous suspension daily and doses of Gantrisin as outlined above. Most of these patients were treated in the time interval immediately preceding the beginning of the controlled series.

The patients in the second group received penicillin in the doses described above plus aureomycin in doses of 50 mg. per kilogram of body weight per twenty-four hours in divided doses intravenously and later orally. Most of these patients were ones in whom an immediate bacteriologic diagnosis was not evident, and the two antibiotics were used in order to cover any eventuality. Four of them also received sulfadiazine, since this drug had been given before admission.

During the period of the present study, another group of 17 patients were treated. These patients had either received a sufficient amount of therapy before they entered the hospital so that they were well on the way to recovery (13 patients) or were treated with aureomycin alone and were too small a group from which to draw any conclusions (4 patients).

The duration of treatment in each of the groups was for seven days after the temperature was essentially normal.

RESULTS

A total of 130 patients have been treated, among whom 11 died (8.5 per cent). In the controlled portion of the study, there were 78 patients, 38 of whom received Gantrisin and 40 penicillin. Eleven patients were in the aureomycin-penicillin group, and 14 were in the penicillin-Gantrisin group.

If the entire group is considered, five clinical or laboratory findings were found to bear a possible relationship to the prognosis, as determined by duration of fever and mental disturbance (the principal criteria which could be used to measure the speed of response). As can be seen in Table I, the age of the patient, a history of convulsions, the presence of mental disturbance on admission, a reduction of spinal fluid dextrose, and an elevation of spinal fluid protein were important in this respect. The following factors were found to be of no significance in relation to speed of recovery: sex of the patient, the number of leukocytes in the cerebrospinal fluid or the peripheral blood, the extent of the rash, the duration of the illness before admission to the hospital, or the height of the fever on admission.

TABLE I. RELATION OF FINDINGS ON ADMISSION TO DURATION OF FEVER AND MENTAL SYMPTOMS

GROUP		NUMBER OF PATIENTS	INTERVAL FROM BEGINNING OF THERAPY UNTIL FEVER PERMANENTLY UNDER 100°F (HR.)	MENTAL STATUS ABNORMAL ON ADMISSION		DURATION OF ABNORMALITY (HR.)
				NUMBER	PER CENT	
All patients		103	79 ± 5.4	39	38	43 ± 4.2
Age						
Under 1 yr.		18	100 ± 18.3	–*	–	
1 to 4 yr.		41	76 ± 7.6	16	39	45 ± 6.0
5 to 9 yr.		19	56 ± 9.0	6	32	22 ± 5.4
10 to 19 yr.		11	94 ± 12.1	8	73	49 ± 7.1
20 yr. and over		14	80 ± 12	8	57	45 ± 8
Convulsions on admission	Present:	12	91 ± 20	6	50	64 ± 14.8
	Absent:	91	78 ± 5.7	33	36	39 ± 4.2
Delirium and/or semicoma† on admission	Present:	39	93 ± 11.3			
	Absent:	37†	67 ± 4.5			
Cerebrospinal fluid dextrose over 20 mg. %		79	68 ± 5.7	17	21	37 ± 2.9
20 mg. % and under		24	97 ± 11.3	22	61	47 ± 7.0
Cerebrospinal fluid protein below 200 mg. %		65	68 ± 5.9	17	27	42 ± 6.2
200 mg. % and over		38	98 ± 10.5	22	58	43 ± 3.3

*Not evaluated in infants.
†No true coma in this series except in the Waterhouse-Friedericksen group.

When a comparison is made of the incidence of the five factors found to be related to speed of recovery in the different therapeutic groups it is found that there is a fairly uniform distribution of cases with no appreciable weighting (Table II).

The results of the various methods of treatment are tabulated in Table III. Only one of the 103 patients died. This patient was in the penicillin group. He was a 45-year-old man who was found lying in the street by the police. In spite of his hyperpyrexia, because he was not completely comatose nor in shock, he was not placed in the therapeutic group reserved for suspected fulminating infections; however, he died one and one-half hours after admission and one hour after a single penicillin injection. Even at autopsy no exact reason for the rapid demise was apparent, since there was no adrenal hemorrhage and practically no cerebral or meningeal involvement. There was, however, pulmonary edema, and it seemed likely that the extreme hyperpyrexia precipitated a sudden pulmonary edema.

In other respects the results of treatment with penicillin seemed to be at least as good, and probably superior to, the other therapeutic regimen in the controlled group. As seen in Table III, the duration of fever was significantly shorter in patients who received penicillin. In addition, the mental changes were less prolonged, although not significantly so. Penicillin alone was slightly better than aureomycin plus penicillin or Gantrisin plus penicillin in the doses used.

As seen in Table IV, in the instances in which the cerebrospinal fluid was examined, the changes in response to therapy were quite similar in the various

TABLE II. DISTRIBUTION OF CLINICAL FINDINGS IN DIFFERENT THERAPEUTIC GROUPS

THERAPEUTIC GROUP	AGE OF PATIENTS					OCCURRENCE OF CONVULSIONS BEFORE TREATMENT		DELIRIUM OR SEMICOMA ON ADMISSION		CEREBROSPINAL FLUID			
										DEXTROSE		PROTEIN	
	UNDER 1 YR.	1-4 YR.	5-9 YR.	10-19 YR.	20 YR. AND OVER	PRESENT	ABSENT	PRESENT	ABSENT*	OVER 20 MG. %	20 MG. % OR UNDER	UNDER 200 MG. %	200 MG. % OR OVER
Gantrisin	4	15	8	5	6	5	33	13	25	23	14	21	15
Penicillin	9	17	5	4	5	5	35	18	22	21	17	23	16
Penicillin and aureomycin	2	5	2	0	2	1	9	4	7	8	2	6	4
Penicillin and Gantrisin	3	4	4	2	1	1	13	4	10	9	1	8	3

*Includes all infants under one year in whom mental status could not be reliably evaluated.

TABLE III. COMPARISON OF RESULTS IN TREATMENT OF PATIENTS WITH MENINGOCOCCIC MENINGITIS WITH GANTRISIN, PENICILLIN AND ANTIBIOTIC COMBINATIONS

TREATMENT GROUP	NUMBER OF PATIENTS	DURATION OF FEVER OVER 100° F. (HOURS)	NUMBER OF PATIENTS WITH DELIRIUM OR SEMICOMA	DURATION OF MENTAL ABNORMALITY (HR.)
Gantrisin	38	91 ± 13	13	49 ± 7.9
Penicillin	40*	50 ± 6.5	18	40 ± 5.7
Penicillin plus aureomycin†	11	78 ± 12	4	72 ± 8.5
Penicillin plus Gantrisin	14	63 ± 10	4	48 ± 8.5

*One patient died—see Table V.
†Four patients received sulfadiazine in addition.

therapeutic groups. The single serious complication occurred in a patient treated with Gantrisin: a 1-year-old girl, who had hemiplegia and was convulsing on admission. The hemiplegia and mental retardation were present on discharge, and on the last follow-up observation, fourteen months after the cessation of therapy.

Untoward reactions to the drugs were infrequent. Three patients who received Gantrisin had drug fever and rash, but no serious renal lesions or blood dyscrasias occurred. One patient in the penicillin group developed urticaria.

In Table V, the course, treatment, and pathologic findings in the fatal cases are summarized. As can be seen, all of these patients showed a fulminating disease with coma and/or shock on admission. In most cases hyperpyrexia was present (temperatures over 106° F.), and in the majority death occurred within twenty-four hours of onset. In all but two there was a rash of the type usually seen in patients with fulminating meningococcemia, consisting of petechiae, ecchymoses, and large cyanotic areas which ultimately become hemorrhagic, although not as completely as the true ecchymoses. In addition, the large, blotchy cyanotic areas were sometimes present which became erythematous if the circulation improved transiently.

Seven of the patients received large doses of penicillin and Gantrisin. Two patients were not treated, since resuscitation procedures were given priority and they died within a few minutes. One patient received aureomycin and one, in the penicillin group, received penicillin alone.

In none of these patients was there more than temporary success in the attempt to support the circulation by the use of intravenous fluids of various kinds and large doses of adrenal hormones. Six of the patients examined had gross adrenal hemorrhage at autopsy.

The patients in this group are the only ones in the entire series who received any adrenal hormones. Three additional patients, one in the Gantrisin group and two in the penicillin group, were in shock on or shortly after admission to the hospital. In none of these did the patient have a fulminating course with mental changes or the typical rash. All three responded promptly to 250 ml. of plasma, and in none was there any suggestion of adrenal insufficiency.

A study has been made of the direct eosinophil count in 26 of the patients on admission to the hospital. In the case of 18 patients, no eosinophils were found. Among the other 8 patients, the eosinophil counts were as follows: 1

TABLE IV. COMPARISON OF SPINAL FLUID CHANGES AND COMPLICATIONS OCCURRING FOLLOWING TREATMENT OF MENINGOCOCCIC MENINGITIS WITH GANTRISIN PENICILLIN AND ANTIBIOTIC COMBINATIONS

TREATMENT GROUP	SMEAR*		CELLS 50 TO 100% DECREASE	OVER 100% DECREASE	SUGAR		PROTEIN		COMPLICATIONS						
					UNDER 40 MG. %	40 MG. % AND OVER	UNDER 50 MG. %	50 MG. % AND OVER	SPINAL FLUID CELLS NORMAL IN		TRANSITORY		PERMANENT		DRUG
	POSITIVE	NEGATIVE							NO. PTS.	DAYS	JOINTS	NERVES	MENTAL	NERVES	TOXICITY
Gantrisin	2	12	1	13	3	11	1	13	6	14 ±2	2	3	1†	1†	3
Penicillin	1	15	3	13	1	15	1	15	13	10.2 ±5	0	3	0	0	1
Penicillin + aureomycin	0	4	0	4	0	4	0	4	5	10 ±4	0	1	0	0	0‡
Penicillin + Gantrisin	1	6	1	6	2	5	0	7	4	11 ±3	0	2	0	0	0

*All cultures negative.

†Mental retardation and hemiplegia in one patient.

‡Three patients had chemical thrombophlebitis from aureomycin.

per cubic millimeter, 2 patients; 2 per cubic millimeter, 1 patient; 11 per cubic millimeter, 2 patients; 22 per cubic millimeter, 1 patient; 88 per cubic millimeter, 1 patient; and 144 per cubic millimeter, 1 patient. The two latter patients were in the early stages of a mild infection. In four patients who were later found to have adrenal hemorrhages at autopsy, the admission eosinophil count was 44, 60, 140, and 250 per cubic millimeter, respectively. In a fifth patient with adrenal hemorrhage who was admitted two hours after the onset and had the typical picture of fulminating meningococcemia, the count was zero, whereas two hours later it had risen to 144. In all of the fatal cases in which eosinophil counts were performed, adrenal hemorrhage was found at autopsy. It is apparent that the eosinophil count gave no more indication of the prognosis than the clinical picture.

DISCUSSION

It seems apparent that within the limitations of the study, penicillin in doses of 1,000,000 units every two hours intramuscularly was at least as effective, and possibly more so, in the treatment of meningococcic infections than Gantrisin in conventional doses.

A possible limitation of the study is that it was done during an endemic period when the only fatalities are in acute fulminating cases and there are practically no deaths from meningitis per se. This was due in part to the fact that most of our patients were in the favorable age groups, namely, between 1 and 40 years of age. Working with a group where the prognosis is so good, one is less likely to be able to demonstrate small differences in therapeutic efficiency. Within this limitation, however, the results obtained seemed to indicate that penicillin in the doses used is excellent therapy. It is apparent that aureomycin adds nothing and perhaps detracts when added to penicillin. Smaller doses of penicillin used with Gantrisin seem to be slightly more effective than the latter alone, but may not be as effective as large doses of penicillin.

The choice of Gantrisin as the sulfonamide used in this study is open to some question, since it has not received the large-scale testing to which sulfadiazine has been subjected. During the past six years it has been our experience that Gantrisin has accomplished everything which one would expect from sulfadiazine. In addition, during this time, we have had no renal complications among well over 100 meningitic patients, an experience which is in considerable contrast to the relatively high incidence of renal involvement from sulfadiazine in the meningitic patient. Since Gantrisin was so superior in this latter respect and seemed of equal therapeutic benefit, it was chosen by us for this comparative study.

An unanswered question is whether the action of penicillin in these large doses would be improved upon or made worse by the use of sulfonamides in addition. From the poor results obtained when this combination was used in the patients with fulminating infections, it is apparent that combination therapy is ineffective in this group, which constitutes the major therapeutic problem.

Our completely negative results on the helpfulness of the adrenal hormones and blood products, in addition to the chemotherapeutic agents in the fulminating infections, is somewhat in contrast to the general impression one gathers

TABLE V. CLINICAL COURSE, TREATMENT AND PATHOLOGIC

AGE (YR.) RACE AND SEX	DURATION OF ILLNESS BEFORE ADMISSION (HOURS)	MENTAL STATUS ON ADMISSION	DEGREE OF SHOCK* ON ADMISSION				FEVER ON ADMISSION	DURATION AFTER ADMISSION TO DEATH	TYPE OF RASH	
			0	+	++	+++			TYPICAL† FULMINATING	PURPURIC
4 W. M.	11	Coma and convulsion				×	107	8 hr.		×
5/12 W. F.	10	Coma				×	104	2 hr.	×	
3/12 W. M.	18	Coma and convulsion			×		103	6 hr.	cyanosis only	
10 W. F.	12	Coma			×		108	26 hr.	×	
3 W. F.	12	Coma				×	106.2	2½ hr.	×	
2 W. M.	12	Coma				×	Not done	15 min.	×	
5 W. F.	14	Coma				×	107	3 hr.	×	
1 N. M.	10	Coma				×	105	30 min.	×	
3 W. M.	4	Coma				×	106.4	10 hr.	×	
24 W. F.‡	6	Coma	×				106	1 wk.		×
45 N. M.	Unknown	Semicoma	×				107	2 hr.		×

*Shock + = low blood pressure, cyanosis and tachycardia.
++ = blood pressure unobtainable, cyanosis and tachycardia.
+++ = only femoral pulse obtainable, or pulse unobtainable.

†See text for description.

‡Pregnancy, seven months.

§Necrosis of pituitary gland.

‖Pulmonary edema.

FINDINGS IN PATIENTS DYING OF MENINGOCOCCAL INFECTION

HEMATEMESIS	CHEMOTHERAPY	SYMPTOMATIC THERAPY	AUTOPSY: ADRENALS: MICROSCOPIC FINDINGS	AUTOPSY: ADRENALS: GROSS HEMORRHAGE	AUTOPSY: BRAIN: EDEMA	AUTOPSY: BRAIN: ENCEPHALITIS	AUTOPSY: MENINGITIS: MICROSCOPIC	AUTOPSY: MENINGITIS: GROSS
+	4,000,000 U. penicillin I. M. and I. V. 4 Gm. Gantrisin I. V.	750 c.c. plasma 250 c.c. blood 70 c.c. cortical extract, I. V.	+	Focal microscopic	++	++ Hemorrhagic	+	+
+	2,000,000 U. penicillin I. V. 2 Gm. Gantrisin I. V.	250 c.c. plasma 50 c.c. cortical extract	+	+	±	0	±	0
0	2,000,000 U. penicillin I. M. 2 Gm. Gantrisin I. V.	750 c.c. plasma 30 c.c. cortical extract	+	–	+	0	–	++
+	11,000,000 U. penicillin I. V. 9 Gm. Gantrisin I. V.	750 c.c. plasma 900 c.c. blood 1,500 c.c. saline 800 mg. cortisone, I. M.	+	+	+	0	±	0
+	2,000,000 U. penicillin I. V. 2 Gm. Gantrisin I. V.	250 c.c. plasma 250 c.c. blood 200 mg. cortisone, I. M. 5 mg. Doca	+	+	+	0	±	0
0	None given in time	125 c.c. plasma artificial resp.	+	+	+	0	±	0
+	Aureomycin 200 mg. I. V.	250 c.c. plasma 250 c.c. blood Cortical extract I. V. 200 c.c. 100 mg. cortisone, I. M.	+	++	Not done			
0	Not given in time	Artificial resp. unable to complete cut down	+	+	+	+	±	0
+	4,000,000 U. penicillin I. V. and I. M. 4 Gm. Gantrisin I. V.	Cortical extract 50 c.c. I. V. Doca 5 mg. I. M Cortisone 200 mg. I. M. Neosynephrine IV drip 500 c.c. plasma 500 c.c. blood	+	–	+	0	–	–
0	90,000,000 U. penicillin I. V. and I. M. Benemid p.o. 50 Gm. Gantrisin I. V. and I. M.	Cortisone, 300 mg. a day 1,500 c.c. blood	+	–	+	0	–	+§
0	1,000,000 U. penicillin I. M.	0	+	0	±	0	0	0‖

from reports of individual recoveries which have appeared in the literature. Some of this difference can be explained on the basis of which patients are considered to be in this group. As outlined, we have used rigid criteria and have had little success in the treatment of patients selected according to these criteria. As reported above, patients who are less ill, although in shock, have been treated much more successfully.

SUMMARY AND CONCLUSIONS

In an alternate case study, 40 patients with meningococcic meningitis were treated with sodium or potassium penicillin, 1,000,000 units every two hours intramuscularly, and 38 patients were treated with Gantrisin in the commonly employed dose. One patient in the penicillin group died one hour after the first dose. The duration of fever over 100° F. rectally in the remaining 39 penicillin patients was 50 ± 6.5 hours, as compared to 91 ± 13 hours in the Gantrisin group. Similarly, the durations of mental change were 40 ± 7 hours and 49 ± 7.9 hours after the start of penicillin and Gantrisin therapy, respectively.

Eleven patients who were treated with aureomycin plus penicillin, because of the nature of prior treatment or uncertainty as to the diagnosis at the start of therapy, had fever for 78 ± 12 hours and mental changes 72 ± 8.5 hours.

Similarly, another group of 14 patients who received penicillin in lower doses, plus Gantrisin, had fever for 63 ± 10 hours and mental changes 48 ± 8.5 hours.

A group of ten patients selected in the admitting office as having fulminating infections were treated with various specific agents, chiefly penicillin in large doses plus Gantrisin, as well as multiple supportive measures. All of these patients expired.

During the time of the study a total of 130 patients were treated, among whom 11 (8.5 per cent) died.

The direct eosinophil count was usually zero in patients who were moderately to severely ill with meningococcic infections. It was sometimes normal or moderately depressed in patients who had fulminating disease or who were only mildly ill.

It is concluded that penicillin in large doses is a satisfactory therapeutic agent for the average patient with meningococcic meningitis and can be used in lieu of sulfonamides when desired.

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LABORATORY METHODS

A SIMPLE, ACCURATE, AND RAPID METHOD FOR THE QUANTITATIVE DETERMINATION OF DEXTRAN IN BLOOD AND URINE

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INTRODUCTION

IN CARRYING out clinical investigation on the use of dextran solutions as plasma substitutes, the need for a simple, rapid, and accurate method of determining the dextran concentration in serum and urine arose. This laboratory originally used the anthrone-sulfuric acid method but this proved rather unsatisfactory. The method was time consuming, required many chemical manipulations, and, in spite of observation of the strict precautions specified,[1-3] reproducible and accurate results could not consistently be obtained.

Since alcohol precipitates dextran from a water solution, it was thought that a turbidimetric method for the quantitative determination of dextran would be feasible. This proved to be the case, and the method and its clinical application are therefore described herein.

METHOD

A. *Technique.*—One milliliter of the solution of unknown dextran content is pipetted into a 125 ml. Erlenmeyer flask. Ten milliliters of 95 per cent ethyl alcohol is added from a Schellbach automatic zero burette while swirling the flask. The resulting turbid solution is then immediately poured into a test tube cuvette and read in the Coleman Junior 6A spectrophotometer against a blank of 1.0 ml. of water and 10.0 ml. of 95 per cent alcohol. The turbid solution must be read as soon as possible after the addition of the alcohol, since it was found that there is a rapid decrease in optical density with time. (This is apparently due to aggregation of the suspended precipitate into larger particles, or to agglutination of the particles to the wall of the flask, or both. It was found expeditious to have all of the solutions being analyzed pipetted into the flasks and to have one member of the group add the alcohol and pass the flasks to the other member who poured the turbid solution into the cuvette and recorded the readings). To ensure accuracy all determinations were done on duplicate aliquots.

B. *Concentration of Alcohol Giving Maximum Precipitation of Dextran.*—The concentration of alcohol giving the maximum precipitation of dextran was determined by the addition of ethyl alcohol of varying concentrations to a dextran solution of given concentration. In Fig. 1 it is seen that no precipitation of dextran occurs below an alcohol concentration of 40

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per cent. Above this concentration precipitation increases rapidly. The maximum optical density, and, therefore, the maximum precipitation of dextran, occurs when the resulting concentration of alcohol in the mixture is between 80 and 86 per cent, or when the original alcohol strength is 88 to 95 per cent. For convenience the 95 per cent alcohol was used throughout.

C. *The Wave Length Giving Maximum Light Absorption.*—The maximum light absorption was determined on dextran solutions of three different concentrations, 0.5, 1.0, and 2.0 mg. per milliliter by taking readings at 10 mμ intervals from 400 to 700 mμ. In Fig. 2 it is seen that the maximum absorption of a suspension of dextran in alcohol occurs at the lower end of the spectrum. There is a low plateau between 420 and 460 mμ, and the midpoint of this plateau, 440 mμ, was therefore taken as the wave length at which maximum absorption occurs. All readings were made at this setting.

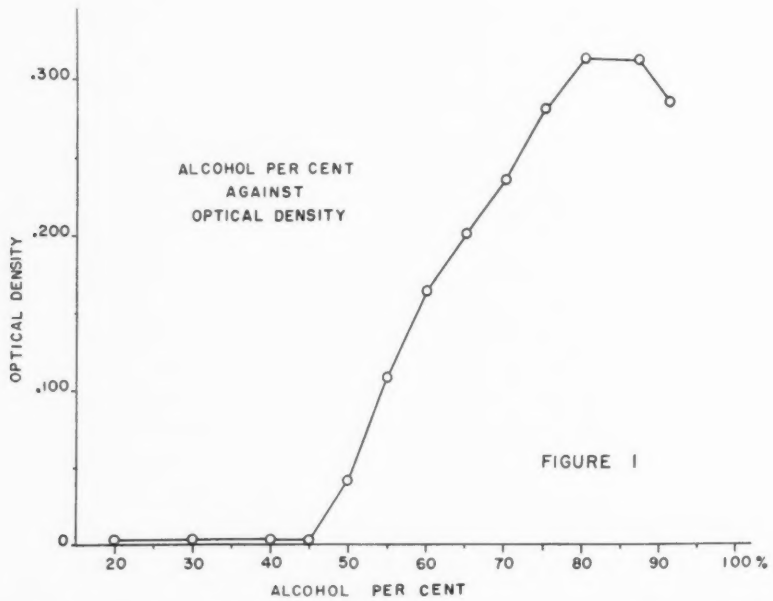

Fig. 1.— Ten milliliters of alcohol are added to 1.0 ml. of solution containing 1.5 mg. of dextran. The maximum precipitation of dextran occurs with 88 to 95 per cent alcohol, or a final alcohol concentration of 80 to 86 per cent.

D. *The Relation Between Optical Density and Dextran Concentration.*—The optical densities were determined on a series of solutions made up to contain 0.1, 0.2, 0.3, ... 8.0, 9.0, and 10.0 mg. of dextran per milliliter. The optical densities determined in the turbid alcohol mixtures were plotted against the respective concentrations taken, and the resulting graph is shown in Fig. 3. Each point on the graph represents the average of three determinations at the given concentration. It will be noted that the curve is sigmoid in shape; however, the segment between 0.3 and 2.2 mg. per milliliter is a straight line. From 2.2 to 10.0 mg. per milliliter the curve slopes progressively away from the linear segment.

For purposes of clinical application of the relationship between optical density and dextran concentration, either of two methods may be applied. Dilutions of serum and urine may be chosen so that their dextran concentrations fall in a given range and then compared with a standard within this range. Or a curve may be constructed from standard dilutions to cover the whole range of concentrations expected in the experiment, and the dextran concentrations of the unknowns determined on this curve from their

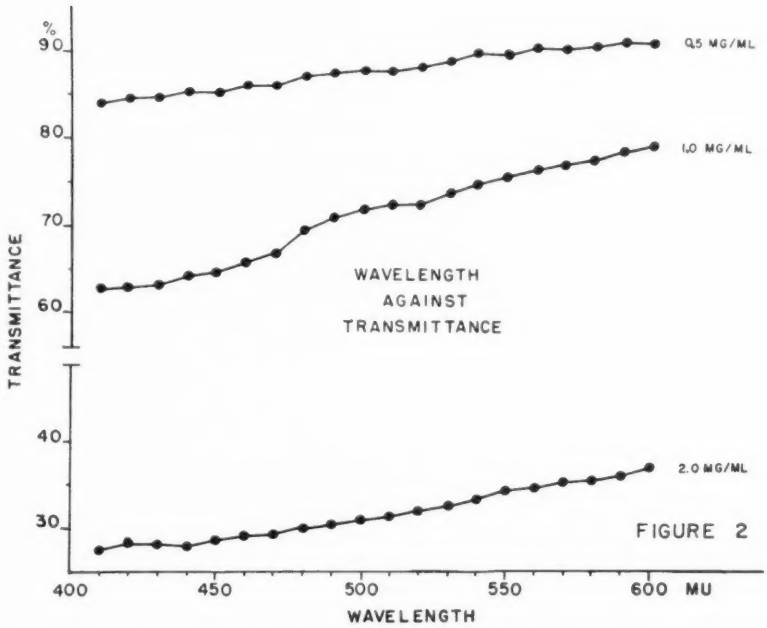

Fig. 2.—The transmittance of a dextran suspension in alcohol. Ten milliliters of 95 per cent alcohol to 1.0 ml. of solution containing 0.5, 1.0, or 2.0 mg. of dextran, with readings at 10 mμ intervals. The maximum absorption is at the lower end of the spectrum.

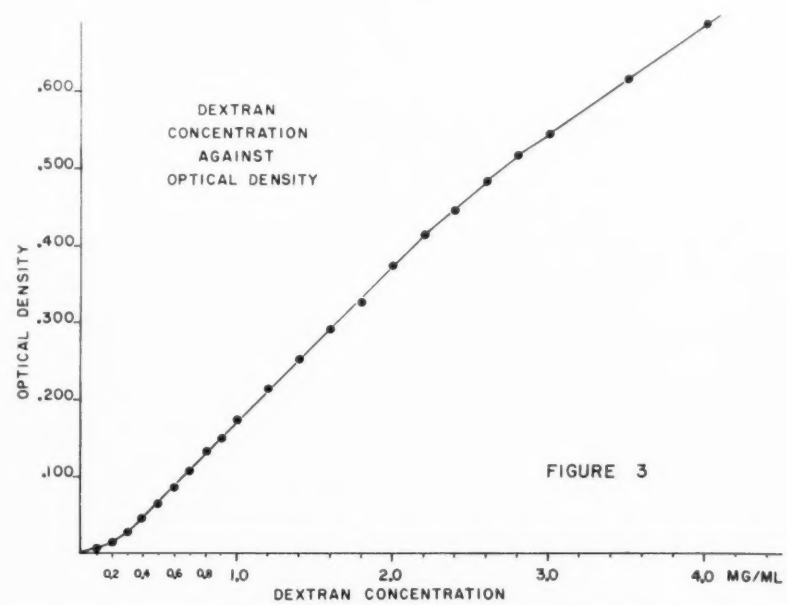

Fig. 3.—The curve of the relation between dextran concentration and optical density is sigmoid in shape. The segment between 0.3 and 2.2 mg. per milliliter is a straight line. Above the latter concentration the optical density increment is progressively less with increasing concentration.

corresponding optical density readings. Should the dextran concentration in the sample be such that the reading is outside the most accurate range of the galvanometer (below 10 and above 90 per cent transmittance), the sample volume may be varied so that the reading will fall within the accurate range.

CLINICAL APPLICATION

A. *The Determination of Dextran in Serum.*—The protein in the serum containing dextran is precipitated with 5.0 ml. of 5 per cent trichloroacetic acid. After centrifugation a 1.0 ml. aliquot of the protein-free supernatant is taken, 10.0 ml. of 95 per cent alcohol added, and the resulting turbid solution read in the spectrophotometer. The concentration of dextran in the aliquot is read from the previously constructed curve, and the concentration in the serum calculated by applying the appropriate dilution factor.

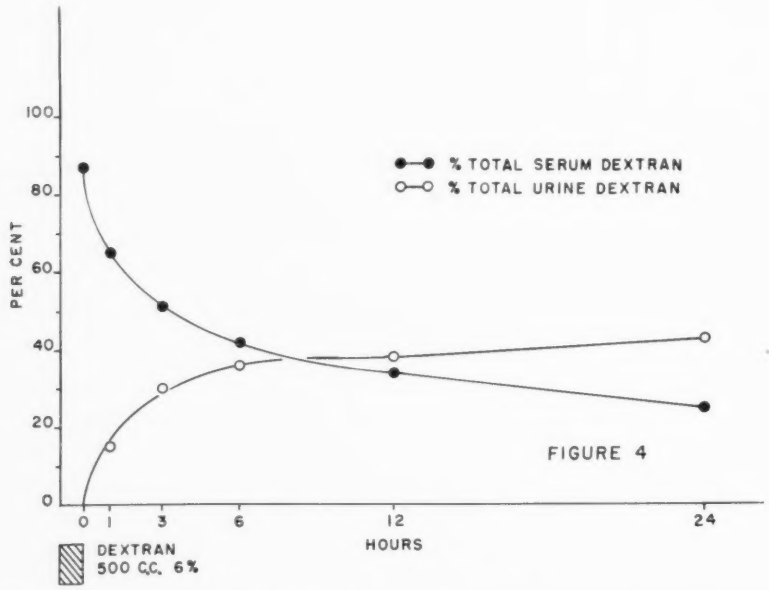

Fig. 4.—Normal patient given infusion of 500 ml. of 6 per cent dextran in normal saline. Blood plasma volumes, urine volumes, and dextran concentrations are determined at the intervals noted, and total dextran as per cent of the amount given calculated. The curves are smooth and the values in keeping with the molecular weight spectrum of the dextran used.

In the clinical experiments the serum dextran concentration ranged from 12.0 to 1.0 mg. per milliliter. The volume of serum taken was therefore varied so that the protein-free filtrate would contain enough dextran to give a reading between 10 and 90 per cent transmittance. Thus, with a serum concentration between 12.0 and 6.0 mg., 0.5 ml. of serum is taken; between 6.0 and 3.0 mg., 1.0 ml. of serum is taken; and below 3.0 mg., 2.0 ml. of serum is taken.

B. *The Determination of Dextran in Urine.*—Clinically the concentration of dextran in urine is five to ten times as great as in the blood serum at corresponding time intervals after an infusion of dextran. The urine is therefore diluted one to ten before the analysis is carried out. This is done with 5 per cent trichloroacetic acid rather than with water for the reason given below.

Two factors interfere with the determination. These are the urinary crystals (oxalates, urates, etc.) and the color of the urine. The former are largely removed by allowing the urine to stand overnight in the refrigerator, and then centrifuging the

supernatant. One milliliter of the clear urine is then diluted with 9.0 ml. of 5 per cent trichloroacetic acid and 1.0 ml. aliquots taken for the determination. When the dextran concentration in the urine is below 3 to 4 mg. per milliliter, 2.0 ml. of urine are taken and 8.0 ml. of trichloroacetic acid used. The acidification keeps practically all of the crystalloids remaining in the urine in solution on the addition of alcohol, and these, therefore, do not interfere with the turbidimetric reading of the dextran precipitate.

The color of the urine varies not only among individuals but from specimen to specimen in the same individual. Even after the addition of the alcohol to the urine, which gives a final dilution of one to one hundred, considerable error is introduced by the urinary chromogens in all but the lightest-colored specimens. To eliminate this error the alcohol-urine mixture is read, not against the stock water-alcohol blank, but against a blank made up for each individual urine specimen. This consists of 1.0 ml. of the diluted urine and 10.0 ml. of water. Although the unknown then contains 10.0 ml. of alcohol, and the blank 10.0 ml. of water, no error is introduced because water and alcohol have the same transmittance at the wave length used.

RESULTS

A. *Recovery Experiments.*—Recovery experiments were set up to determine the reliability of the method. Three stock sera were taken and portions of each made up to contain from 1.0 to 16.0 mg. of dextran per milliliter (in increments of 1 mg. from 1 to 6, and of 2 mg. from 6 to 16). The reference standard was a water solution of dextran made up in the same concentrations. The mean of the recoveries in the resulting 33 serum samples as per cent of the water standard was 99.6 per cent, with a standard deviation of ± 4.26 per cent. If the 1.0 mg. samples are omitted from the series (at this concentration the method is somewhat inaccurate), the recovery of the remaining 30 samples was 100.2 ± 3.00 per cent.

The recovery from urine was determined by taking ten routine urine specimens varying in color from straw to dark mahogany and making up portions of each to contain 3, 6, 9, 18, and 36 mg. of dextran per milliliter. A reference standard with water was similarly prepared. The recovery in the resulting 50 specimens was 100.5 ± 4.01 per cent.

B. *Duplicate Analysis.*—The reproducibility of the method was determined by analyzing fifty determinations done in duplicate on various sera, and fifty on various urines, obtained in the clinical experiments. The determined serum dextran concentrations in the fifty specimens ranged from 1.5 to 12.0 mg. per milliliter, and the mean agreement between the duplicates was 100.6 per cent, with a standard deviation of ± 2.93 per cent.

The fifty urines analyzed ranged from 1.0 to 50.0 mg. of dextran per milliliter. The mean agreement between the duplicates was 100.0, with a standard deviation of ± 4.16 per cent.

C. *Clinical Use.*—This method for dextran determination was employed in following the blood serum dextran concentration and the urinary excretion of dextran at serial intervals up to twenty-four hours after an infusion of 500 ml. of 6 per cent dextran in twelve patients. The values for the amount of dextran remaining in circulation and for the cumulative amount of dextran appearing in the urine gave perfectly smooth curves in each patient. An example of the type of curve obtained is shown in Fig. 4.

DISCUSSION

The anthrone-sulfuric acid method for the determination of dextran in blood and urine is currently in use in many laboratories. The method is very sensitive, and polysaccharide in concentrations as low as 10 μg per milliliter may be determined. However, the method presents a number of difficulties and possible sources of error. Many chemical manipulations are involved. The anthrone dye is variable in composition. The anthrone-sulfuric acid reagent changes on standing. The heat developed and therefore the color of the reaction mixture varies with the size of the vessel, the rapidity of addition of the reagent to the unknown, and the manner of mixing of the two. Glucose present in protein-free filtrates adds considerable error, particularly when the dextran concentration is relatively low. Lint and filter paper fibers also introduce some error.

In the clinical studies on dextran as a plasma substitute, concentrations below 1.0 mg. per milliliter in serum or urine are not very significant. The sensitivity of the anthrone method is therefore not required in this type of study. By comparison with the anthrone method the turbidimetric method described above is very simple. It requires only two reagents commonly found in every laboratory, 95 per cent ethyl alcohol and trichloroacetic acid. The chemical manipulations are few, and the method is therefore quite rapid. Accurate results are obtainable without any particularly strict precautions. There is no interference in the determination by blood constituents, such as glucose, in the protein free filtrate. The method is applicable to clinical work because it is accurate in the range of concentrations found in blood serum and urine of patients in the twenty-four or forty-eight hours following an infusion of 500 to 1,000 ml. of 6 per cent dextran.

SUMMARY

A turbidimetric procedure for the determination of dextran in serum and urine has been described. It requires only two common reagents, ethyl alcohol and trichloroacetic acid, and few chemical manipulations. It is therefore simple and rapid.

Although not as sensitive as the anthrone method, the described method is applicable to clinical work since it is accurate in the range of dextran concentrations found under clinical conditions. The method has been used in following the physiological effects of dextran infusions in patients with very satisfactory results.

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A METHOD FOR THE DETERMINATION OF FREEZING POINT OF SMALL QUANTITIES OF BIOLOGICAL FLUIDS

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INTRODUCTION

ESTIMATIONS of the osmolar concentration of body fluids from the freezing point, vapor pressure, or other indices provide information of physiologic and clinical significance. However, methods of measurement previously described have not been entirely satisfactory, either because of technical difficulties, lack of sufficient sensitivity of measurement, or because the volume of sample needed for analysis has been larger than is readily available from biologic sources. The method for measurement of freezing point temperature to be described here can be performed with relative ease, precision, and rapidity on small volumes of solution.

APPARATUS

Cooling Bath (Fig. 1).—The cooling bath is a modification of the Hortvet cryoscope.[1] The refrigerant, ethyl ether, is contained in a wide-mouthed, one quart vacuum bottle enclosed in a metal casing.* A tight-fitting stopper is used to close the bottle and support the apparatus immersed in the bath.

Refrigeration is accomplished by evaporation of the ether under reduced tension.† An intake system is indicated at the left of Fig. 1. As shown, room air is drawn under the surface of propylene glycol. This permits a visual estimate of the rate of flow. It also acts to reduce the concentration of water vapor when the room air is moist and thus prevents condensation and icing on the metal bubbler ring. The air outlet system is shown at the right of Fig. 1. This is connected to a vacuum pump. Since relatively little negative pressure is necessary to effect adequate refrigeration, a simple, manually adjusted, faucet type pump may be used. Bubbles rising from the bubbler ring to the surface of the ether bath suffice to prevent thermal layering. A long-stemmed mercury thermometer is immersed in the bath so that the temperature can be maintained at a known, desired level.

A brass jacket designed to provide a snug sheath for the glass freezing tube is immersed centrally in the cooling bath. The upper, open end is adjusted to lie flush with the vacuum bottle stopper. When in use a few drops of alcohol are placed in the bottom of the jacket so that, after introduction of the freezing tube, no air space exists to impede heat exchange between the walls of the tube and the brass jacket.

Freezing Tubes.—Matched Pyrex glass test tubes, 22 by 175 mm. (Corning No. 9820), have been used and found suitable for sample volumes between 2.0 and 4.0 milliliter. Once

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*Universal Vacuum Filler, No. 012, Landers, Frary, and Clark, New Britain, Conn.

†As an alternative to the use of evaporating ether for refrigeration, a small expansion coil can be placed in the vacuum bottle. This is connected in a closed circuit to a Freon compressor unit. Refrigeration may be controlled by adjustable solenoid valves or by automatic thermostatic controls. Brine or a 25 per cent solution of propylene glycol is used for the bath. Under these circumstances the bath fluid can be allowed direct access to the freezing chamber by multiple fenestrations in the wall of the brass jacket.

the sample has been introduced the tube is closed with a No. 3 rubber stopper. This stopper carries the shaft of the sensing element through a central perforation. A metal sleeve, fixed in a second offset perforation, acts as a bearing for the shaft of the agitator. Mixing in the freezing tube is accomplished by the up-and-down motion of a stainless steel washer which encircles the tip of the sensing element and is attached laterally to the agitator shaft. The shaft is coupled at its upper end to the arm of a stirring motor geared to one revolution per second. Interposed between the arm of the stirring motor and the agitator shaft is a damping spring to prevent damage should the agitator be thrust forcibly in contact with the bottom of the freezing chamber.

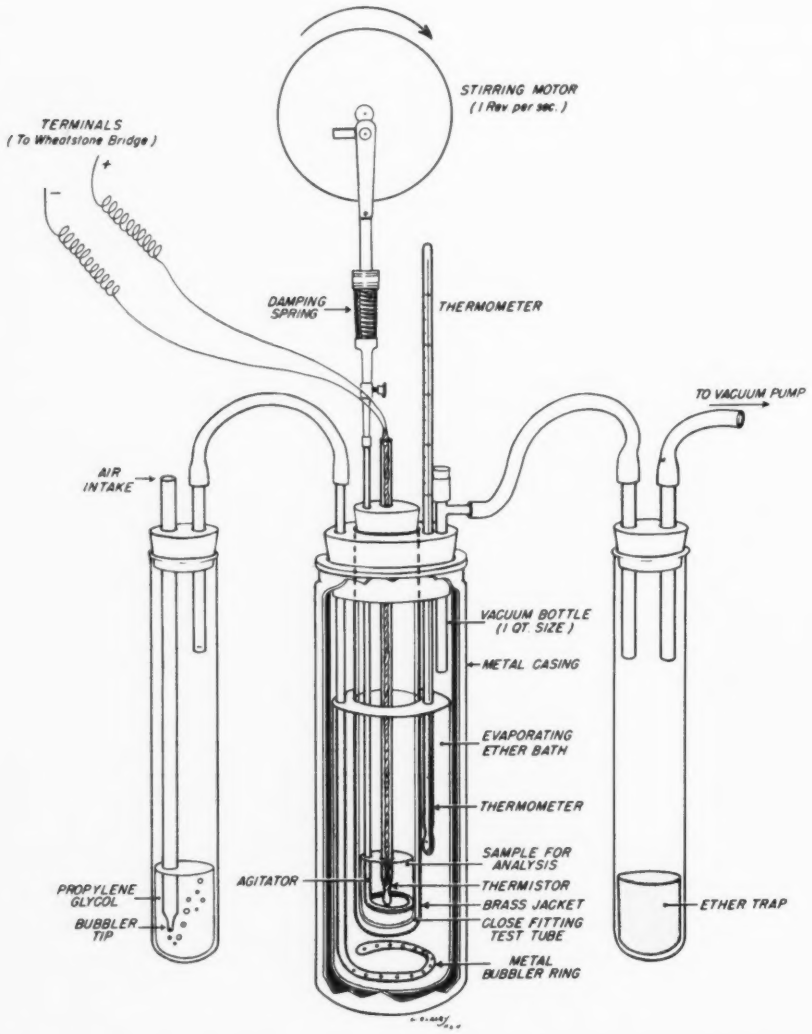
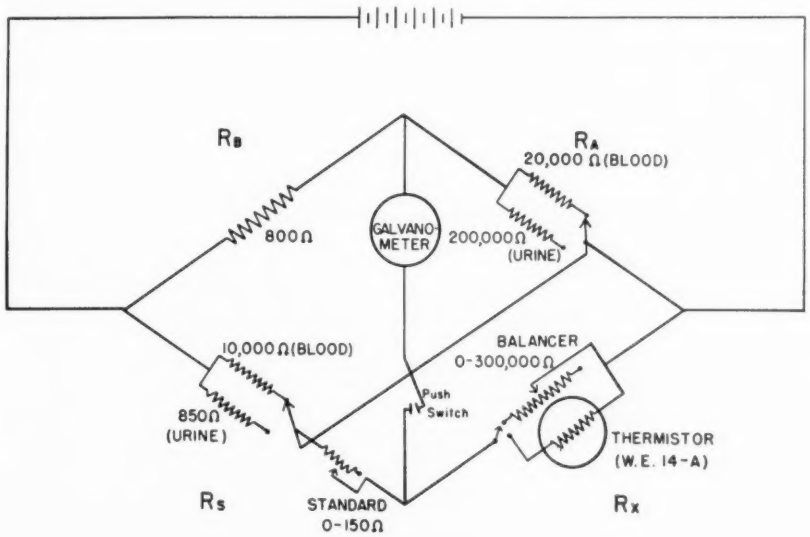


Fig. 1.—*Cooling system for freezing point determination.* In the model shown, ether evaporated under slight negative pressure is used as the refrigerant. A brine or propylene glycol bath cooled by an immersed Freon expansion coil may be substituted for the ether, as suggested in the text. Controls for maintaining the bath temperature constant are not shown. The temperature of the bath can be read from the long-stem mercury thermometer and adjustments made either by manual regulation of the vacuum pump or by an automatic thermostatic control.

Sensing Element.—The sensing element is a glass-enclosed thermistor (Western Electric Co., No. 14-A). The heat-sensitive tip of the element is approximately 2 mm. in diameter. The terminals emerge from a glass stem 6 cm. in length and 1.5 mm. in diameter. These thermistor elements can be mounted for convenience of handling in glass tubing 7 mm. in outside diameter, wall thickness 1 mm., and 20 cm. in length. These mounting tubes are tapered to a diameter of about 2 mm. at one end. A water-tight and slightly flexible seal can be made between the shoulder of the thermistor element and the tapered end of the glass tubing by means of a 0.5 cm. length of No. 6-F soft rubber catheter. This rubber sleeve is



CIRCUIT DIAGRAM OF FREEZING POINT APPARATUS

Fig. 2.—*Circuit diagram of Wheatstone bridge.* Electric current is furnished by a 6-volt heavy-duty storage battery. The sensing element (thermistor) forms leg *Rx* of the bridge. A protective circuit through an adjustable resistance is also provided in this leg and is used to balance the bridge when the resistance of the sensing element is beyond the range of compensation by the variable resistance in leg *Rs*. A small portion of the total resistance of leg *Rs* is in the form of a variable rheostat (Standard). The 10 turn "Helipot" (the Helipot Corporation, South Pasadena, Calif.) equipped with a "Duodial" scale makes possible readings from 1 to 1,000, with subdivisions of each unit to quarters. Simultaneously switched, alternate circuits through fixed, precision resistances are available in legs *Rs* and *Rx*. These permit measurement of resistance changes in the sensing element either over a range of 5° C., with an accuracy of ±0.005° C., or over a range of 0.5° C. with an accuracy of ±0.0005° C. The former circuit is employed chiefly for determinations on fluids varying greatly in concentration such as urine. The latter circuit is particularly suited for determination on blood or other fluids where small variations in concentration are important.

slipped over the tip of the thermistor onto the adjacent portion of its shaft. Then the thermistor leads and shaft are introduced into the tapered end of the glass tubing to the position of the rubber sleeve. Using soap as a lubricant, the sleeve and thermistor shaft are pushed up into the glass tubing until just the tip of the thermistor and a short collar of rubber protrude. After washing and thoroughly drying, the mounted thermistor tip can be coated with liquid, waterproof plastic (Ambroid), or Spar varnish. The terminals of the thermistor should be fixed at the upper end of the glass tube so that no strain is transmitted to the fragile glass stem. Finally, the mounted thermistor is pushed through the central perforation of the No. 3 rubber stopper used to close the freezing chamber. It is advanced until the tip lies 3 mm. from the bottom of the freezing chamber when the stopper is in place.

Instrumentation.—The Wheatstone bridge circuit used to measure the electrical resistance of the sensing element is shown in Fig. 2. Alternate circuits in legs *Ra* and *Rs* permit

temperature measurements either over a range of 5.0° C. with the error of ±0.005° C., or over a range of 0.5° C., with an error of ±0.0005° C. An alternate, dummy resistance is provided in leg *Rx* so that the bridge can be maintained in balance when the thermistor is at room temperature. The galvanometer is a direct-current, mirror type with a sensitivity of 0.01 microamperes per millimeter, coil resistance 1,100 ohms, and external critical damping resistance of 6,000 ohms.

Standard Solutions.—A set of standard solutions of known concentration should be prepared, since the freezing point of a solution of unknown concentration is found by interpolation between the freezing points of two or more standards. Solutions of potassium chloride prepared according to the data given in Table I are satisfactory for this purpose. The freezing points of these solutions are distributed through the range 0.000 to -1.860° C. in temperature steps of 0.086° C. Expressed in terms of effective osmolar concentration (Table I, column IV) such standards have a constant difference of 100 mosM/Kg.H_2O one from the next in order of increasing or decreasing concentration. If kept stoppered and free from contamination, they are stable indefinitely at room temperature.

TABLE I. STANDARD SOLUTIONS

I STANDARD NUMBER	II CONCENTRATION OF KCl IN WATER (MM/KG.H_2O)	III FREEZING POINT* (DEGREES CENTIGRADE)	IV EFFECTIVE OSMOLAR CONCENTRATION (MOSM/KG.H_2O)
1	0	0.000	0
2	53.5	-0.186	100
3	108.5	-0.372	200
4	164.1	-0.558	300
5	220.1	-0.744	400
6	276.3	-0.930	500
7	332.7	-1.116	600
8	389.3	-1.302	700
9	446.4	-1.488	800
10	503.3	-1.674	900
11	560.3	-1.860	1,000

*Calculated from data of The International Critical Tables[2] according to the formula:

$$\text{Log } X = (\text{Log } y \div f) - K$$

where X = Freezing point depression in degrees C.
y = Concentration of solute in millimoles per kg. of water.
f = Slope factor for solute; f for KCl = 1.020.
K = Constant; value for KCl solutions = 2.425.

PROCEDURE

Preparatory Steps.—Initially, the cooling bath temperature should be brought to -3.0° C. and stabilized at that point. At the same time, the current should be turned on in the Wheatstone bridge circuit and a period of twenty minutes allowed for warm-up and electrical stabilization. The protective circuit in leg Rx rather than the circuit through the thermistor is used during this period to obtain balance. Three milliliter volumes of standard solutions and of the fluids to be analyzed are pipetted into clean, dry freezing tubes. After stoppering, these tubes should be stored in a refrigerator at about 4.0° C.

Procedure of Freezing.—For adjustment of the galvanometer and the initial determination a tube of standard solution is selected which has a freezing point lying near the center of the range expected for the freezing points of the unknown solutions. The thermistor and agitator are introduced into this tube and centered by adjustment of the rubber stopper against the glass rim of the tube. Then the tube is placed in the receptacle of the cooling bath, the agitator shaft coupled to the stirring motor, and the motor turned on. If the standard solution has been precooled in the refrigerator to 4.0° C., only about sixty seconds further cooling in the bath is necessary before its temperature reaches 0° C. or below. At this time the circuit through the thermistor is substituted for the protective circuit in leg Rx of the Wheatstone bridge. The progress of further cooling is indicated by increasing deflection of the galvanometer indicator. After approximately two minutes the temperature

of the standard solution will be close to −3.0° C. and well below the freezing point of most biological fluids. Crystallization is now initiated by lifting the agitator shaft so that the impetus of the stirring motor is taken up momentarily by the damping spring. Just before the stirring arm is pushed by the stirring motor to the lowest point of its cycle, the agitator shaft is released. The spring-impelled plunge of the agitator results in sudden, violent turbulence of the supercooled, standard solution. Rapid crystallization with release of heat follows. This heat release is indicated by a sudden swing of the galvanometer indicator in the direction opposite to that observed during cooling. Stabilization at the freezing point is complete sixty seconds after initiation of freezing. At this point the galvanometer indicator will remain steady for another minute's time. During this period there is an opportunity to adjust the galvanometer string so that the indicator is in the center of the scale when the rheostat in leg *Rs* is adjusted to the center of its range.

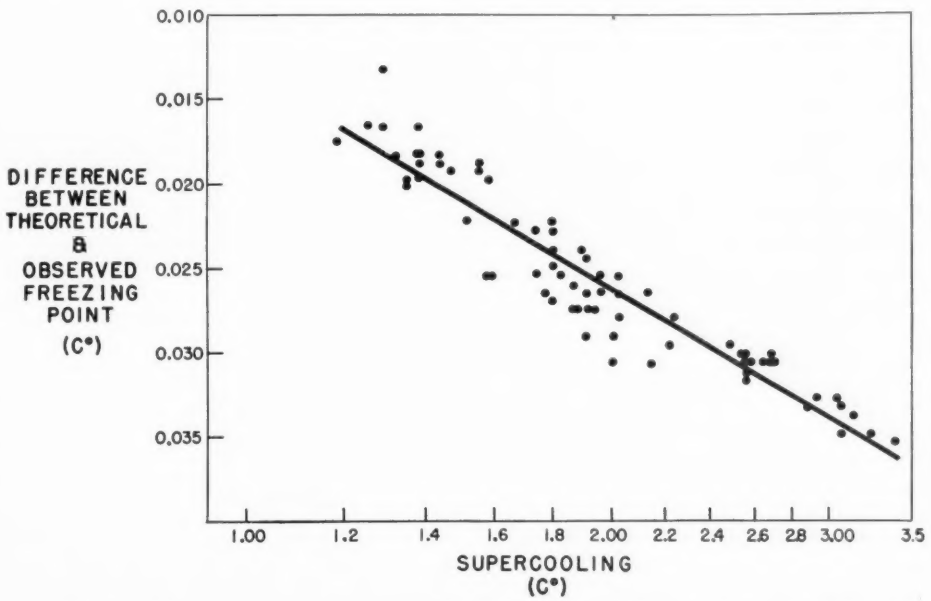


Fig. 3.—*Effect of supercooling on freezing point.* The difference between the theoretical freezing point temperature and the highest temperature observed during freezing of a solution is shown by the scale of the ordinate. The logarithmic scale of the abscissa shows the extent of supercooling occurring before initiation of crystallization. The experimental points describe a straight-line relationship between the coordinates. This relationship may be used to compute the amount by which the observed freezing point temperature is lowered by supercooling.

After the initial freezing, the tube containing the standard solution is removed from the cooling bath and warmed until the last ice crystals have disappeared. Now the freezing procedure is repeated, this time taking note of the amount of supercooling prior to the initiation of crystallization.

When the galvanometer indicator again shows that a constant freezing point temperature has been reached, the indicator is brought to the nul point at the center of the scale by adjustment of the rheostat. The reading of the rheostat scale at this point corresponds to the known freezing point of the standard solution minus the small correction factor for supercooling. The effect of supercooling on the freezing point of a solution is shown by the curve of Fig. 3. The appropriate correction factor for each individual reading is determined from this curve.

Construction of Standard Curve.—The rheostat setting necessary to balance the Wheatstone bridge circuit at the freezing point of the first standard gives the initial point for

construction of a curve relating temperature to the scale of the rheostat. Since the resistance of the thermistor can be considered directly proportional to temperature through the narrow range of freezing points of biological fluids, the above-mentioned curve will be rectilinear and its slope constant for a given thermistor element. Hence the slope of the curve can be found by freezing a second standard solution, the concentration of which is either above or below that of the first. Ordinarily it is advisable to determine the rheostat settings necessary to balance the Wheatstone bridge circuit at the freezing point of three different standard solutions. A graphic plot of these settings against the known freezing points of the standard solutions should yield a straight-line relationship. Nonlinearity indicates inaccuracy of preparation of the standards or improper operation of the thermometry apparatus.

If the apparatus is in use over a protracted period, it is advisable to recheck at intervals of not more than an hour one or more of the points on the standard curve. Changes in room temperature may be responsible for slow changes in the rheostat setting corresponding to the freezing point of a given standard. Since these changes fail to influence the slope of the standard curve, periodic checks on a single standard solution yield the information necessary to correct the entire curve to compensate for this effect.

Determination of Freezing Points of Solutions of Unknown Concentration.—The freezing points of samples of biological fluids or other solutions of unknown composition are obtained by the same technical procedure as has been described earlier with respect to the freezing points of the standard solutions. The rheostat reading which balances the bridge at the freezing point of the unknown is referred to the standard curve relating the rheostat reading to temperature. The freezing point temperature of the unknown solution is found by interpolation on this curve (Fig. 3).

DISCUSSION

The oncotic properties of biological fluids have been investigated by various techniques including freezing point, vapor pressure, membrane diffusion, and electrical conductivity. The same general type of information is given by all of these precedures.

The freezing point determination has a significant advantage over other methods because of the simplicity of the technique involved. The main disadvantages of this method have been referable to the mercury thermometers ordinarily used as sensing elements. The prolonged thermal time constant and large size of the thermometers required for this type of work has necessitated the use of sample volumes larger than are readily available from biologic sources. Furthermore, the best available thermometers are difficult to read with precision greater than 0.01° C. Use of the thermistor as a sensing element results in reduction of the thermal time constant to a matter of a few seconds. The size of this sensing element is so small that sample volumes can be reduced to 2 ml. using the freezing tubes described above. Substitution of tubes of smaller diameter permits even further reduction in sample volumes. Readings accurate to 0.001° C. can be made with a suitable Wheatstone bridge circuit and sensitive galvanometer. The high resistance of the thermistor unit and the small amount of current passing through it make negligible the factor of internal heating of the element. Hence, this system of thermometry overcomes essentially all of the disadvantages mentioned above without adding to the difficulty or time expended in making determinations.

The thermistor unit is not to be recommended without qualification. Different elements do not have identical characteristics, nor does one element retain its individual characteristics indefinitely. Nevertheless, initial calibration and periodic rechecking of the behavior of a given unit is not difficult. Furthermore, the elements are inexpensive.

This method has been in use for four years in our laboratory. During this period several hundred measurements have been made on samples of blood, tissue juice, and urine. The results of these will be reported in separate communications.

SUMMARY

A resistance thermometry method for determining the freezing point of biologic fluids is described. The advantages of the method are its simplicity, accuracy, and adaptability for use with quantities of solution less than 5 ml. in volume.

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THE HAMSTER AS A VEHICLE FOR THE DEMONSTRATION OF PATHOGENICITY OF ACTINOMYCES BOVIS

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DIFFERENTIATION of the potentially pathogenic strains of *Actinomyces bovis* and the saprophytic actinomyces-like strains ("anaerobic diphtheroids"[1-2]) isolated from pathologic material presents frequently a serious and troublesome problem in the diagnostic laboratory. This difficulty is due not only to the close similarity of some of these microorganisms in morphologic and cultural characters but more so to the lack of knowledge of their pathogenic properties.[3] These properties cannot easily be determined at the present time, since there is no reliable animal test.

Pure cultures of *A. bovis*, as shown by many investigators,[4-7] have low, if any, pathogenicity for the usual small laboratory animals. Meyer and Verges[2] reported that 94.6 per cent of young, male, albino mice developed progressive actinomycosis following the intraperitoneal injection of *A. bovis* cultures mixed with gastric mucin, and that only 31.9 per cent developed lesions when the same cultures were suspended in saline. They also noted that with few exceptions the mucin did not enhance the invasiveness of "anaerobic diphtheroids," and in such instances the lesions differed from those produced by *A. bovis*.

In 1947 Hazen, Martin, and Dogan[8] and Hazen and Martin[9] reported, apparently for the first time, the use of young male hamsters (3 to 4 weeks old) in pathogenicity tests with *A. bovis* and saprophytic actinomyces-like strains. It was found later that intraperitoneal injections of a single heavy saline suspension of *A. bovis* into the hamster resulted in severe lesions in which typical actinomycotic granules with clubs were present.[10] As a result of these observations, the comparative study[8-9] of strains of *A. bovis* and the saprophytic actinomyces-like strains ("anaerobic diphtheroids") was continued. The objective was to investigate further the pathogenicity for the hamster of a large number of such strains and to determine whether the strains having pathogenic properties possessed certain biochemical properties unlike those of the saprophytic strains. Such differential criteria, if found, might serve as an aid in identification. The results of this study will be reported at a later date. The results in a limited number of young male hamsters of single intraperitoneal injections of saline suspensions of pure cultures of *A. bovis* only are reported at this time.

MATERIALS AND METHODS

Eight pure cultures of *A. bovis*, isolated from pathologic materials, were used. All isolates were of comparatively recent origin with the exception of culture No. 43395 which had been isolated at the central laboratory in 1943 and kept in the dry state. The seven

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other cultures had been maintained since isolation on beef heart infusion agar containing 5 per cent defibrinated horse blood.[11a] All of the cultures showed the morphologic and cultural characters of the species *A. bovis* generally accepted as the incitant of actinomycosis in man. Male hamsters three to four weeks old were employed.

Culture suspensions in physiologic salt solution were used for the intraperitoneal injections. They were prepared as follows: Meat infusion broth[11b] containing 1 per cent

TABLE I. RESULTS IN HAMSTERS AUTOPSIED FOUR TO SIX WEEKS FOLLOWING A SINGLE INJECTION OF A LIVING CULTURE OF ACTINOMYCES BOVIS

SOURCE OF CULTURE	NUMBER INJECTED	NUMBER INFECTED	REACTION	MICROSCOPIC FINDINGS: MOIST PREP.	MICROSCOPIC FINDINGS: GRAM STAIN	CULTURE OF LESION FOR A. BOVIS
No. 4769—Pus with sulfur granules from abscess of jaw.	2	2	Severe	Not made	microorganisms	+
No. 49296—Sputum from patient with history of asthma of many years. Diffuse fibrosis of both lungs.	2	1	Severe	Not made		+
No. 49297—Pus with sulfur granules from fatal abdominal actinomycosis.	6	6	2 Severe and extensive 4 Severe	Many granules with clubs		+
No. 50155—Bloody exudate with granules without clubs from mass on right thigh. Lesion 5 yrs.	4	2	Severe	Granules with clubs	branching	+
No. 5105—Sputum from undiagnosed pulmonary infection.	4	2	Severe and extensive	Granules with clubs		+
No. 5272—Bloody exudate on swab from lesion on lower jaw.	4	4	2 Severe and extensive 2 Limited	Granules with clubs	pleomorphic	Not made +
No. 5273—Culture for identification isolated from pus from sinus in neck.	2	1	Severe and extensive	Granules with clubs		+
No. 43395—Culture maintained in dry state for several years. Isolated from abscess in neck and shoulder.	4	3	1 Severe and extensive 2 Severe	Granules with clubs	Gram-positive	+

Severe and extensive reaction: Multiple encapsulated abscesses of from 1-15 mm. in diameter throughout the abdominal cavity; the abscesses contained purulent material of soft or cheesy consistency and showed the presence of granules with clubs. The infection extended through the parietal peritoneum and abdominal wall or through the diaphragm with a resultant sinus.

Severe reaction: Essentially same as above but infection did not extend beyond abdominal cavity.

Limited reaction: Small encapsulated abscess containing purulent material in which granules with clubs were demonstrated.

glucose in 10 ml. amounts in test tubes (18 by 150 mm.) was inoculated with several colonies picked from blood agar plates or growth scraped from blood agar slants; the tubes were incubated anaerobically in the Fildes-McIntosh jar for about one week. The broth cultures were always prepared in duplicate. The clear broth was removed from each culture sediment and 1 ml. of physiologic salt solution added; the two suspensions were combined and 1 ml.

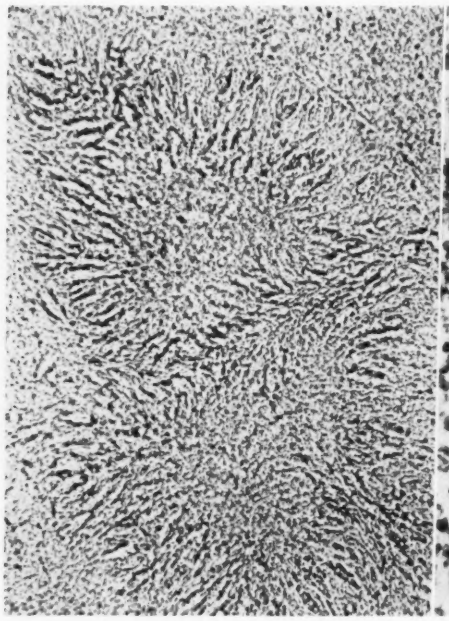

Fig. 1.

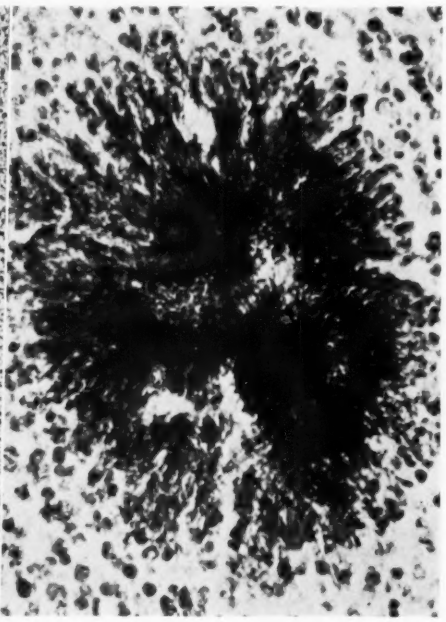

Fig. 2.

Fig. 1.—Two unstained granules with clubs in pus from encapsulated abscess in hamster injected with *A. bovis* No. 5273; magnification ×450.

Fig. 2.—Histologic section of encapsulated abscess from hamster inoculated with *A. bovis* No. 50155, showing granules with clubs and mass of filaments stained by Hotchkiss-McManus technique; magnification ×900.

Fig. 3.—A film preparation of pus from abscess in hamster injected with *A. bovis* No. 50155, showing pleomorphic branching microorganisms stained by Gram's method; magnification ×1200.

was injected intraperitoneally into each of two hamsters. The animals were observed for palpable lesions for four to six weeks, after which they were sacrificed and examined for gross and microscopic evidence of infection.

RESULTS

Twenty-one (75 per cent) of twenty-eight hamsters given a single intraperitoneal injection of eight cultures of *A. bovis* and sacrificed four to six weeks later showed definite evidence of infection.

Nineteen of the twenty-one animals developed severe infections, showing extensive abscess formation throughout the abdominal cavity, in five of which the infection had extended through the parietal peritoneum and abdominal wall with a resultant sinus. The infection spread to the outer skin surface in four of the animals and in one extended through the diaphragm to the pleural space. Two of the twenty-one animals injected with the same culture (No. 5273) showed definite evidence of infection but of a milder type.

Seven (25 per cent) of the twenty-eight hamsters showed no gross evidence of infection, although it will be seen that in each case the duplicate animal injected simultaneously with the same culture did show severe reactions.

Granules with clubs were found in pus from every abscess where a microscopic examination was made (Figs. 1 and 2).

Gram-positive pleomorphic branching microorganisms were demonstrated in stained film preparations of pus from every animal in which abscesses were present (Fig. 3), and *A. bovis* was isolated from the pus in every instance where cultural examinations were made.

SUMMARY AND CONCLUSIONS

The reactions of twenty-eight young (three to four weeks old), male hamsters to intraperitoneal injection of a single dose of eight strains of *A. bovis* are described. The results of the microscopic and cultural studies of pus collected from abscesses at autopsy are also presented.

Each one of the eight strains of *A. bovis* was found to have pathogenic properties for the young hamster. There is a possibility that the pathogenicity of the microorganism was increased by the medium (meat infusion broth) carried over in the inoculum used for injection of the hamsters.

Seventy-five per cent of the hamsters presented reactions resembling those of clinical actinomycosis, the presence of granules with clubs in the pus being a constant feature. Twenty-five per cent failed to show gross evidence of infection, although the duplicate hamsters, injected simultaneously with the same cultures, developed severe infections. It is possible that these failures were due to technical error, resulting from the large number of clumps of microorganisms in the inoculum.

The results suggest that, of the small laboratory mammals, the young male hamster is the animal of choice for the study of the pathogenic properties of *A. bovis*.

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PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

Twenty-Fifth Annual Meeting

Chicago, Ill., Nov. 7 and 8, 1952

ABSTRACTS—Concluded

73. A SEVENTEEN-YEAR FOLLOW-UP STUDY ON THE CLINICAL AND HEMATOLOGIC RESULTS OF TOTAL THYROIDECTOMY IN A CASE OF POLYCYTHEMIA VERA

A Clinical Experiment

Louis R. Limarzi, M.D., Jerome T. Paul, M.D., and Lindon Seed, M.D. (By Invitation), Chicago, Ill.

A case of polycythemia vera on which total thyroidectomy was performed because of the anemia and decreased blood volume that is found in some cases of spontaneous myxedema was presented before the Society in 1936 (*Proc. Cent. Soc. Clin. Research,* **9**: 23, 1936) and again in 1937 (*Proc. Cent. Soc. Clin. Research,* **10**: 18, 1937). The original blood values were hemoglobin 21.5 Gm., erythrocytes 8.62 million, hematocrit 79 per cent, and blood volume 10,700 c.c. After the existence of myxedema for two years the blood values dropped to hemoglobin 11.5 Gm., erythrocytes 3.18 million, hematocrit 33 per cent, and blood volume 3,790 c.c. The basal metabolic rate was minus 28, and the blood cholesterol 250 milligrams. The patient was relieved of all her former symptoms. The myxedema and macrocytic anemia were controlled with thyroid extract. During the next four years she was reasonably free of her preoperative symptoms. However, she then began to experience recurrence of headaches and dizziness, and examination of blood showed hemoglobin 17.0 Gm. and erythrocytes 7,800,000. Her symptoms were relieved by repeated venesections. Since 1943 she has not required any further venesections, and has taken thyroid extract intermittently. Recurrent thrombophlebitis of the legs and neck has been treated by anticoagulants.

The patient is now 68 years of age, and presents a marked splenomegaly, palpable liver, and a dry, pale, scaly skin. There is a Grade II systolic murmur at the apex, roentgen ray evidence of gross cardiac enlargement, and an electrocardiogram shows left heart strain pattern. The hemoglobin is 10.6 Gm., erythrocytes 3,700,000, hematocrit 36 per cent, leukocytes 3,500, reticulocytes 4 per cent, and platelets 365,000. The blood smear shows an occasional immature granulocyte and nucleated red blood cell. The bone marrow is essentially normal. The basal metabolic rate is plus 20 and blood cholesterol 198 milligrams. Radioactive iodine studies with a tracer dose of 50 mc. show an uptake of 1 per cent and a conversion ratio of 3 per cent. She is able to pursue her routine duties, although at times the myxedematous symptoms become pronounced because of the failure of the patient to adhere to the dosage of thyroid extract recommended.

74. BONE MARROW STUDIES IN INFECTIOUS DISEASE

JOHN LOUIS, M.D. (BY INVITATION), MARK H. LEPPER, M.D. (BY INVITATION), AND LOUIS R. LIMARZI, M.D., CHICAGO, ILL.

WITH THE TECHNICAL ASSISTANCE OF
MARGARET L. FLORENCE AND ADELLE M. BLAZAICH

The blood and bone marrow were studied in 107 patients in different stages of acute infectious disease, and the results compared with those in normals. The diseases included rubeola 28, scarlet fever 23, varicella 20, mumps 14, rubella 9, poliomyelitis 7, and pertussis 4. The ages ranged from 6 months to 25 years. The most characteristic marrow finding was a hyperplasia and immaturity of the granulopoietic tissue. This is quantitatively expressed by a maturation index which is obtained from the ratio of immature to mature granulocytic elements. The index for the controls in the same age group was 0.18; whereas in rubeola the index was 0.54, scarlet fever 0.54, varicella 0.50, mumps 0.48, pertussis 0.44, rubella 0.40, and poliomyelitis 0.28. The granulocytic-erythrocytic ratio was elevated: rubeola 11.6, scarlet fever 10.6, pertussis 9.2, varicella 6.9, rubella 6.4, mumps 6.2, and poliomyelitis 3.5, as compared with normal 2.94. The average white blood cell counts were: rubeola 8,300 (3,000 to 10,000), rubella 7,700 (3,500 to 15,200), varicella 9,000 (4,600 to 17,200); mumps 9,400 (4,700 to 17,200), scarlet fever 11,800 (4,500 to 22,000), poliomyelitis 12,000 (6,600 to 20,000) and pertussis 16,700 (8,400 to 29,600). There was no apparent relationship between the peripheral white count and granulocytic activity in the marrow.

Marrow eosinophilia was present in scarlet fever and rubella, and correlated closely with the blood eosinophilia. Marrow lympocytosis was present only in poliomyelitis. The plasma cells were increased in the blood and marrow of rubeola, rubella, varicella, and mumps. In scarlet fever the marrow plasmacellular reaction was not deflected in the blood, while in pertussis the reverse relationship was observed. Erythropoiesis was normal. Increased megakaryopoiesis could not be correlated with the platelet level in the peripheral blood. L.E. cell phenomenon was not observed in defibrinated bone marrow preparations. Histologic sections of marrow particles from 4 cases of rubella and 2 cases of varicella showed no granulomatous lesions or follicles of lymphoid hyperplasia.

75. BRONCHIAL LYSOZYME

THOMAS H. LORENZ, M.D., AND WILLIAM P. DEISS, M.D., MADISON, WIS.

(INTRODUCED BY OVID O. MEYER, M.D.)

An investigation of the occurrence and possible significance of lysozyme in bronchial mucus of subjects with and without bronchopulmonary disease was undertaken.

Specimens of mucus (approximately 10 ml.) were obtained by direct aspiration from the bronchial tree in 4 subjects. Two of these were spinal-bulbar poliomyelitis patients who had tracheotomies but no evidence of intrinsic bronchopulmonary pathology. These were considered as experimental controls. The third had extensive bronchiectasis proved by bronchography, and the last patient had severe intrinsic bronchial asthma of ten years' duration.

Assay of bronchial secretions for lysozyme was done using a method recommended by Armour & Co., based on a modification of methods of Goldsworthy and Florey and Boasson.

Lysozyme was found to be present in the secretions of all 4 subjects. Furthermore, it was observed that the concentration of this enzyme was similar in the 2 controls (10,800 units and 9,000 units), but distinctly elevated in the asthmatic (30,000 units) while decreased in the bronchiectasis patient (1,080 units).

Although these differences may be attributable to the consistency of the secretions studied, the results of this study suggest that further investigation of the role of lysozyme in the bronchial secretions in health and disease is indicated.

76. THE EFFECT OF PITUITARY GROWTH HORMONE ON THE ACETOACETATE SYNTHESIS OF THE LIVER

WILLIAM D. LOTSPEICH, M.D., CINCINNATI, OHIO

(INTRODUCED BY STANLEY E. DORST, M.D., AND RICHARD W. VILTER, M.D.)

A study was made of the effect of the administration of crystalline anterior pituitary growth hormone on the synthesis of acetoacetate by the liver. Several different growth hormone preparations were administered to adult female rats in sufficient quantities to affect a definite acceleration in body growth. The acetoacetate production of liver slices from treated and untreated rats was measured in vitro. It was found that under these conditions the liver slices from the growth-hormone-treated animals produced significantly greater quantities of acetoacetate than liver slices from their untreated pair fed controls. From histochemical studies it was found that the "ketotic" livers of the growth-hormone-treated rats showed a marked depletion in glycogen content. In contrast to this, there was no change in liver fat content that could be correlated with the growth-hormone treatment. It is suggested that the pituitary growth hormone causes, either directly or indirectly, an acceleration in the rate of fatty acid metabolism in the liver, and that this process is directly associated with the mechanisms which furnish the energy for the protein synthesis of body growth. It is further suggested that the ketosis of uncontrolled diabetes mellitus might be the result of an aberration in the normal mechanism whereby the growth hormone causes the liver to produce increased quantities of ketones during growth.

77. THE CONVERSION OF N^{15}-LABELED MESOBILIRUBINOGEN TO STERCOBILINOGEN BY FECAL BACTERIA

PAUL LOWRY, M.D. (BY INVITATION), NEWELL R. ZIEGLER, M.D. (BY INVITATION), AND C. J. WATSON, M.D., PHD., MINNEAPOLIS, MINN.

Previous studies in this laboratory indicated that mesobilirubinogen is intermediary in the bacterial reduction of bilirubin to stercobilinogen in the human colon. Subsequently Baumgärtel has claimed that fecal bacteria convert bilirubin to mesobilirubin and hence directly to stercobilinogen, mesobilirubinogen (urobilinogen) according to this concept being formed only by cellular dehydrogenases in the liver. Lemberg and others have accepted this belief which would drastically modify current concepts of the enterogenous character of urobilinogen. Since the keystone of Baumgärtel's hypothesis is the supposed inability of fecal bacteria to convert mesobilirubinogen to stercobilinogen, we have studied the effect of such organisms on crystalline mesobilirubinogen.

A meat extract, yeast enriched broth was inoculated with feces from a normal individual and incubated at 37° C. After twelve hours, either bilirubin

or mesobilirubinogen was introduced. At the end of forty-eight hours, urobilinogen or stercobilinogen was extracted and isolated as the corresponding crystalline urobilin or stercobilin. The fecal bacteria rapidly reduced bilirubin to stercobilinogen. The speed of conversion in such experiments has thus far made it impossible to detect an intermediary mesobilirubinogen. It is clear, however, that fecal cultures under the same conditions readily convert mesobilirubinogen to stercobilinogen. Depending on the amount of mesobilirubinogen added, the crystalline stercobilin isolated exceeded from three to eight times the stercobilin which had been present in the fecal medium.

This finding was confirmed in the most convincing manner by means of N^{15}-labeled mesobilirubinogen. This was obtained by Zinkham reduction of N^{15}-labeled crystalline bilirubin, isolated from human feces after administration of aureomycin and N^{15} glycine. This method will be described elsewhere. N^{15} mesobilirubinogen (0.594 atom per cent excess) was incubated in a fecal culture from which crystalline stercobilin ($\alpha \frac{d}{20^\circ C.} = -4000$; abs. max. 4925 m$\mu$), 0.555 atom per cent excess N^{15}, was subsequently isolated. Thus, a reduction of mesobilirubinogen to stercobilinogen had clearly taken place.

Since fecal bacteria reduce both bilirubin and mesobilirubinogen to stercobilinogen, and since we have repeatedly been able to crystallize mesobilirubinogen from human feces, there is little reason to postulate an extraintestinal site for its formation, or to doubt its intermediary relationship between bilirubin and stercobilinogen.

78. POSTURAL EFFECTS IN TETRALOGY OF FALLOT

PAUL R. LURIE, M.D., INDIANAPOLIS, IND.

(INTRODUCED BY KENNETH G. KOHLSTAEDT, M.D.)

In eleven patients with tetralogy of Fallot, quiet standing and/or passive foot-down tilting consistently decreased arterial oxygen saturation as measured by oximeter or arterial puncture. Return to the horizontal reversed these effects. Blood pressures during these observations were unchanged. Both tight abdominal binders and elastic bandaging of the extremities mitigated the effects of standing and tilting, without affecting blood pressure.

One cooperative patient was subjected to cardiac catheterization during which the table was tilted foot-down 20, 45, and 70 degrees. Cyanosis progressively increased. Severe dyspnea occurred at 70 degrees. Prior to the onset of dyspnea, the following observations held: the systemic and pulmonary artery flows fell while the per cent venoarterial shunt remained constant. Systemic blood pressure remained constant. Pulmonary ventilation rose while oxygen consumption per minute remained constant.

Our data suggest an explanation for all of the postural effects upon arterial saturation in tetralogy on the basis of altered venous return and cardiac output dependent upon the effects of gravity. Mixed caval blood saturation diminishes when systemic flow decreases and oxygen utilization remains unchanged. Systemic artery saturation will then parallel mixed caval saturation and therefore systemic flow so long as the pulmonary venous saturation and the per cent venoarterial shunt remain constant. These conditions were obtained in the patient studied.

The postures of predilection in this disease (squatting, knee-chest, prone, or lateral preferred to supine decubitus, legs drawn up when sitting on chair)

ossess in common the factor of facilitating the emptying of the great veins of the lower extremities and abdomen.

The differences in the postural responses of tetralogy from those of other cyanotic congenital cardiac conditions probably lie in two features: (a) absence of cardiac failure which decreases cardiac output and renders patients insusceptible to further creases upon tilting; (b) fixed obstruction of the pulmonary artery as op (to variable obstruction afforded by altered arterioles in such conditions as nenger's complex. In tetralogy of Fallot par excellence there is subnorm coronary flow and increased systemic flow so that the amount of blood sent far from the heart and subjected to the effects of gravity is much gr nan in other conditions.

79. TENFOLD INCREASE IN BIOLOGICAL ACTIVITY OF CHORIONIC GONADOTROPHIN WHEN MIXED WITH PLASMA THEREBY ALLOWING ITS MEASUREMENT IN THE PLASMA OF MEN RECEIVING THE HORMONE

WILLIAM O. MADDOCK, M.D., PHD., AND ROBERT B. LEACH, M.D. (BY INVITATION), DETROIT, MICH.

Although chorionic gonadotrophin has been used therapeutically for over twenty years, determinations of blood levels in patients receiving the hormone have not been reported, apparently because blood levels were expected to be too low to measure. Therefore, when surprisingly high plasma levels of hormone were by chance observed in the plasma of a man receiving chorionic gonadotrophin, observations were extended to the following 7 patients: 3 hypogonadotrophic eunuchoids, 2 men having panhypopituitarism, and 2 men having hypergonadotrophic hypogonadism. Each patient received 5,000 I.U. of chorionic gonadotrophin in 2 c.c. of water intramuscularly three times weekly for two weeks to three months. Chorionic gonadotrophin levels were determined in immature rats using uterine and ovarian weights as end points. Before therapy no hormonal activity was detected in any plasma sample. Plasma obtained twenty-four hours after the initial dose contained hormonal activity equivalent to 1 to 4 I.U. per cubic centimeter, or, in terms of estimated total plasma volume, 4,000 to 9,000 I.U. Levels as high as this, or higher, were maintained during therapy. Three to four days after the last injection plasma levels of hormone were still approximately one-half of those found during therapy. Five to seven days after treatment plasma hormone content had fallen to barely detectable levels.

The observation that the total measured hormonal activity of the plasma often exceeded the dose administered was explained by finding that the addition of plasma (obtained from untreated individuals) to an aqueous solution of chorionic gonadotrophin resulted in a five- to tenfold increase in potency when administered to the assay rats by either subcutaneous or intraperitoneal injection. Incubation of the hormone-plasma mixture at 37° C. for four hours further increased the activity to twice that of the unincubated mixture. Boiled human plasma, rat plasma, egg white, and polyvinylpyrrolidone also produced significant augmentation. Since no augmentation was observed when plasma and hormone were administered at separate sites in the assay rats, the most likely mechanism of augmentation appears to be prolongation of absorption from the site of injection.

80. NATURE OF THE RELATION OF ASCORBIC ACID DEFICIENCY TO THE METABOLISM OF FOLIC ACID COMPOUNDS

CHARLES D. MAY, M.D., AGNES HAMILTON, M.A. (BY INVITATION), AND CHARLES T. STEWART, M.D. (BY INVITATION), IOWA CITY, IOWA, AND MINNEAPOLIS, MINN.

Folic acid deficiency and megaloblastic anemia have been found by the authors to develop regularly as a complication of scurvy in monkeys. The present experiments were devised to determine whether ascorbic acid deficiency interfered with the normal metabolism of folic acid compounds or if there is merely an increased requirement for folic acid in scurvy. The effect of ascorbic acid deficiency on the following phases of folic acid metabolism was determined: (a) the bacterial synthesis of folic acid compounds, (b) the absorption of folic acid compounds from the intestine, (c) the liberation of folic acid from its conjugates by the action of conjugase, (d) the conversion of folic acid to folinic acid (citrovorum factor) in vivo, and (e) the comparative potency of folic acid and folinic acid in treating megaloblastosis in scurvy.

It was found that the conversion of folic acid to folinic acid in vivo might be less efficient in scurvy, but ascorbic acid was not necessary for this conversion to take place. All other phases of the metabolism of folic acid compounds were found to proceed normally in scurvy. Marked deficiency of folic acid compounds did not accompany the experimentally induced ascorbic acid deficiency until signs of scurvy had become well advanced. Folic acid deficiency in scurvy appears to be due to an increased requirement in that disease rather than to a disturbance in the metabolism of folic acid compounds specifically induced by ascorbic acid deficiency.

81. ACQUIRED HEMOLYTIC ANEMIA AND VIREMIA

HERBERT R. MORGAN, M.D., ROCHESTER, N. Y.

Whole blood specimens were obtained from six patients with acquired hemolytic anemia and specimens of spleen from three patients following splenectomy. All but two of these patients gave evidence of hemolytic activity at the time the specimens were taken. Erythrocytes from six of the patients exhibited autoagglutination and positive Coombs antiglobulin tests.

Suspensions were prepared from the whole clotted blood and spleen fragments by grinding with alundum in nutrient broth. After the addition of 100 units per milliliter of penicillin and of streptomycin, these suspensions were injected into embryonated eggs by the allantoic sac, amniotic sac, and yolk sac routes. Following incubation, evidence for growth of viruses was sought by examining the chick embryo and by hemagglutination tests on allantoic and amniotic fluids from these eggs with chicken erythrocytes. Three serial blind passages were carried out in each instance, and the fluids or yolk-sac tissues from the final passage inoculated on the chorioallantoic membranes of embryonated eggs as an additional test for the presence of viruses.

These virus isolation techniques, which should be adequate for such agents as Newcastle disease, influenza, mumps, and psittacosis viruses, and probably suitable for herpes and certain pox viruses, gave no evidence for the presence of viruses in the blood of these patients, as has been reported by other investigators (Moolten and Clark) in previous studies.

82. NOREPINEPHRINE—CLINICAL RESULTS AND THE EFFECT ON GLOMERULAR FILTRATION RATE IN THE TREATMENT OF SHOCK

JOHN H. MOYER, M.D., JAMES M. SKELTON, M.D. (BY INVITATION), AND LEWIS C. MILLS, M.D. (BY INVITATION), HOUSTON, TEXAS

Measures aimed at increasing the blood volume are usually satisfactory for the treatment of shock. However, there are a number of patients in whom the shock state progresses in spite of the restoration of the normal blood volume. In these patients, additional fluid administration is usually of no value, and may actually lead to congestive heart failure. This is not only true of surgical patients in whom the hypotensive state has existed for prolonged periods of time, but even more true of patients in whom the shocked state has resulted from nonsurgical causes, particularly in those conditions associated with low cardiac output. It is inescapable that the sooner one returns the blood pressure to levels adequate for perfusion of vital organs the more likely the patient is to recover. Therefore, an agent is needed which will consistently raise the blood pressure and which has a short duration of pressor activity so that the blood pressure can be closely regulated from minute to minute. Norepinephrine, primarily a peripheral vasoconstrictor, has been found to be effective in this respect.

Norepinephrine was used by intravenous infusion in the treatment of 18 patients in severe shock following myocardial infarctions, 14 patients with shock due to overwhelming infection, 15 patients with shock secondary to medications, and 28 patients with so-called "irreversible" surgical shock. A satisfactory and immediate pressor response was obtained in all but 2 patients. However, the underlying cause of the shock, whether it was medical or surgical, had to be corrected in order for the patient to recover. Raising the blood pressure from normotensive levels to hypertensive ones occasionally produced supraventricular arrhythmias. No evidence of cardiac effect was noted when the blood pressure was raised from shock levels to normotensive ones. Increasing the blood pressure from normotensive to hypertensive levels produces vasoconstriction in the kidney. However, when previously reduced from the shock state, glomerular filtration rate progressively improved as the blood pressure returned toward normal. A particularly striking increase was noted when the shock was secondary to reactions to medication. It is concluded that norepinephrine, by continuous infusion or a similar vasopressor agent of short duration, is the method of choice for the treatment of shock in patients in whom the blood volume is normal or increased.

83. SOME STUDIES CONCERNING THE PATHOLOGIC PHYSIOLOGY OF THE MAMMALIAN BLOOD PLATELET UTILIZING P^{32}-TAGGED RABBIT PLATELETS

JOHN F. MUELLER, M.D., CINCINNATI, OHIO

(INTRODUCED BY RICHARD W. VILTER, M.D.)

An isotopic labeling technique has been devised for the study of the blood platelet. Purified, unagglutinated platelet suspensions are prepared from rabbit blood by repetitious centrifugation. Upon incubation with P^{32} in vitro specific activity of high order is acquired by the platelet. This can be shown by repeated washings and correlation of the platelet count with the radioactivity.

The kinetics of assimilation were studied, since little is known concerning the metabolism of the platelet. The three phosphorous compartments, acid-soluble, lipid, and organic, were explored. It was found that, if incubation were limited to two to three hours, most of the P^{32} resided in the acid-soluble portion and that the isotope moved out with great ease, thus nullifying usefulness. However, if incubation were continued throughout the night, most of the P^{32} was tightly bound in the organic compartment. Further incubation of these platelets failed to dislodge the P^{32}. Therefore, tracer studies were possible with such radioactive platelet suspensions.

Homologous radioactive platelet suspensions were injected into the ear vein of rabbits, and heart blood was obtained for Geiger counting at varying intervals. Ten such experiments to date have indicated that less than 5 per cent of the injected activity remains after two hours, and only about one-third of the activity can be recovered immediately. The animals were sacrificed, specimens of various organs removed, ashed, and counted for radioactivity. The latter is recovered in the organs high in reticuloendothelial activity, spleen, liver, lung, and bone marrow in decreasing concentration.

These results suggest that under the conditions of the experiment transfused platelets remain in the recipient a very short time, and apparently are filtered out by the reticuloendothelial system. Since these results are at variance with reported life span of human platelets, viability of radioactive platelets must be established. These platelets appear normal morphologically with brilliant cresyl blue. Endogenous respiration does occur in them as measured in a Warburg apparatus. The serum prothrombin time of platelet-poor plasma reverts to normal when they are added. Clots prepared from purified fibrinogen will retract upon addition of treated platelets.

84. A STUDY OF URORENNIN EXCRETION IN PATIENTS WITH PERNICIOUS ANEMIA

EDWIN G. OLMSTEAD, M.D. (BY INVITATION), AND JOHN S. HIRSCHBOECK, M.D., MILWAUKEE, WIS.

Sylvest demonstrated in 1949 that the milk coagulating ability (urorennin activity) of urine could be measured accurately by first activating a fasting morning urine specimen at pH 3 for one hour at 37.5° C. and then buffering this urine to a pH of 4.9 and adding 5 ml. of milk to this mixture and timing the coagulation of this milk with a stop watch. This time compared with the time of coagulation of milk by a standard pepsin solution and by means of a simple formula, the unknown quantity of urorennin could be determined in the urine. The results were stated in rennin units (R.U.), one rennin unit equalling the milk coagulating ability of 0.1 of a milligram of pepsin.

Sylvest was the first to test urorennin activity in pernicious anemia. In 30 patients with proved pernicious anemia, he found that the excretion of urorennin was less than 0.3 R.U. per 10 ml. of fasting morning urine, while only three of a control group of sixteen patients excreted less than 0.3 R.U. per 10 ml. of fasting morning urine. Loss of urorennin activity was attributed to chronic atrophic gastritis.

Fifty patients known to have pernicious anemia in remission were studied for urorennin activity. Fifty "normal" patients in the pernicious anemia age range (40 to 90 years) were also studied. These patients had no evidence of hematologic, gastrointestinal, or renal involvement. Of the 50 patients with pernicious anemia studied for urorennin excretion, the mean excretion of urorennin was 0.098 R.U. per 10 ml. The minimum excretion was 0.00 R.U. per 10 ml. The maximum excretion was 0.50 R.U. per 10 ml. Of the fifty normal

patients in the pernicious anemia age range, the mean urorennin excretion was 1.72 R.U. per 10 ml. The minimum excretion was 0.10 R.U. per 10 ml. The maximum excretion was 4.04 R.U. per 10 ml.

If the urorennin excretion in a fasting morning urine specimen is over 0.5 R.U. per 10 ml. of urine, it is *extremely* unlikely that this patient is suffering from pernicious anemia. If the urorennin excretion is under 0.05 R.U. per 10 ml. of urine, the patient may or may not be suffering from pernicious anemia, and further diagnostic study is indicated. The urorennin excretion test may be used in place of the gastric analysis in the diagnosis of pernicious anemia.

85. SENSITIVITY OF PATIENTS WITH MYASTHENIA GRAVIS TO TUBOCURARINE AND DECAMETHONIUM

E. W. Pelikan, M.D., J. E. Tether, M.D., and K. R. Unna, M.D., Chicago, Ill.

(Introduced by E. F. Foley, M.D.)

Threshold doses of two muscle relaxants, tubocurarine and decamethonium, were determined in 30 patients with myasthenia gravis and in 22 nonmyasthenic subjects. Drugs were administered by a timed, interrupted, intravenous infusion technique (tubocurarine, 1.5 μg/lb./min.; decamethonium, 0.75 μg/lb./min.). The dose of each drug required to produce paresis of the extraocular muscles in each subject was determined.

Myasthenic subjects were 5.5 times as sensitive to tubocurarine (threshold dose, 5.43 ± 4.19 μg/kg.) as were nonmyasthenic subjects (threshold dose, 24.77 ± 10.85 μg/kg). The effect of threshold doses of tubocurarine on myasthenic patients was to increase the weakness of the muscles affected by the disease.

Five per cent of nonmyasthenic subjects had thresholds to tubocurarine in the range of the mean threshold dose for patients with myasthenia gravis. Similarly, 5 per cent of the patients with myasthenia gravis had thresholds to tubocurarine which were essentially normal.

The threshold dose of decamethonium in patients with myasthenia gravis (5.53 ± 3.18 μg/kg.) was insignificantly different from the threshold in nonmyasthenic subjects (4.29 ± 2.80 μg/kg.). In contrast to tubocurarine, decamethonium in subthreshold doses in patients with myasthenia gravis produced a transient remission of signs and symptoms of the disease before paralysis supervened. The improvement did not persist after termination of the experiment.

All experiments were performed without hazard to the subjects. The effects of threshold doses of tubocurarine were quickly reversed by neostigmine or m-hydroxyphenyl-ethyldimethylammonium bromide (Tensilon).

Pharmacological analysis of neuromuscular function in myasthenia gravis is consistent with the hypothesis that a fundamental aspect of the disease is the presence in the organism of a substance which blocks neuromuscular transmission by a mechanism of action similar to that of tubocurarine.

86. THE ANTIHEPARIN EFFECT OF POLYBRENE

(A Polymer of N,N,N^1,N^1,-Tetramethylhexamethylenediamine and Trimethylene Bromide)

Frederick W. Preston, M.D., Chicago, Ill.

(Introduced by Smith Freeman, M.D., PhD.)

Polybrene administered intravenously to dogs corrected the hypocoagulability of blood caused by the administration of heparin. Approximately 7.5

mg. of Polybrene neutralizes 10 mg. of heparin (1,000 U.S.P. units) within five minutes after intravenous injection. Heparin did not alter the blood coagulation time if given within thirty minutes after an injection of Polybrene (in the ratio of 7.5 mg. of Polybrene to 10 mg. of heparin). Decreasing heparin neutralization effect was detectable one-half to eight hours after Polybrene administration, and no definite effect was apparent after eight hours.

Polybrene is similar to protamine sulfate and toluidine blue in that it neutralizes heparin in vivo and in vitro and acts as an anticoagulant in vitro in high concentrations.

Hypocoagulability of the blood was produced in 11 dogs by exposure to 350 to 400 roentgens of total body x-radiation. Definite prolongation of the three tube Lee-White clotting time was apparent in all animals eight to sixteen days after exposure. In no case was there an appreciable lowering of prolonged coagulation time following the intravenous administration of Polybrene in doses of 1 to 2 mg. per kilogram.

Peptone shock was produced in 3 dogs by the intravenous administration of 10 per cent Bactoprotone solution. Hypocoagulability of the blood to a degree similar to that observed in the irradiated dogs was detected soon after the onset of shock. Polybrene in doses of 1 to 2 mg. per kilogram restored the coagulation time to the control levels in all animals within five minutes after injection. No toxic effects attributable to Polybrene were noted in any animals from the doses used in these studies.

87. A CONTINUOUS RECORDING DETERMINATION OF EARLY DISAPPEARANCE OF IODINATED (I^{131}) HUMAN SERUM ALBUMIN FROM CIRCULATING BLOOD

W. H. Pritchard, M.D., W. J. MacIntyre, PhD. (By Invitation), T. W. Moir, M.D. (By Invitation), and S. R. Inkley, M.D. (By Invitation), Cleveland, Ohio

A technique of continuous recording of the dilution of iodinated human serum (I^{131}) albumin by the circulating blood volume was accomplished in 8 patients free of heart disease, edema, or severe anemia. Whole blood assays on freely flowing arterial blood were determined by leading the blood from an indwelling femoral arterial needle through a plastic tube, past the crystal face of a shielded scintillation counter, and returning it into an antecubital vein. By using an appropriate scaler, computer, and an Esterline Angus recorder, a continuous recording of the early (one to two hours) critical portion of the dilution curve was easily possible. In addition, venous blood samples were drawn at hourly intervals from two to six hours, and whole blood activity was measured to complete the latter part of the curve.

Approximately 100 μc of tagged albumin was used. This dose insured sufficiently high counts for suitable accuracy and rapid recording without being prohibitive of repeated doses. To aid in clot prevention in the tubing system, 50 to 100 mg. of heparin was given intravenously after the arterial needle was in place in some patients. All recorded curves were extrapolated to zero time.

It was found that the individual curves of all except one patient exhibited a single semilogarithmic decline over the four- to six-hour study period following initial mixing. Each curve, however, showed a different rate of fall with a range from 2 per cent to 12 per cent loss at one hour and from 4 per cent to 23 per cent loss at two hours. No study has been attempted to explain the differences in slopes.

In one patient two separate decline rates were evident, and suggested the mixing of two different intravascular components, i.e., a freely circulating and a more "inaccessible" pool.

The curves also demonstrated that mixing was complete within ten minutes in all patients and that loss of material was neglible at this time, since the maximal loss at ten minutes did not exceed 3 per cent, irrespective of the curve slope.

The implications of these curves relative to the necessity of multiple specimens of blood for single or serial accurate blood volume determinations will be discussed.

88. THE PROTHROMBIN OF THE BABY AT BIRTH

ARMAND J. QUICK, M.D., LUIZ G. MURAT, M.D. (BY INVITATION), AND CLARA V. HUSSEY, M.S. (BY INVITATION), MILWAUKEE, WIS.

The ease with which hypoprothrombinemia develops in the newborn infant is well recognized but poorly understood. In a series of cases in which the prothrombin concentration was determined in the blood of the mother, of the cord, and of the baby immediately after birth, it was found that when the mother received vitamin K (Synkavite) the prothrombin time of all three bloods was normal. When no vitamin K had been given, the prothrombin of the baby's and the cord blood was invariably low, and was even slightly subnormal in the mother's blood. It was further found that the baby's blood with a normal prothrombin time had a total prothrombin approximately only one-fourth that of adult blood. This suggests that the newborn baby, like the dog and the rabbit, has all of its prothrombin in the free state, which alone is measured by the prothrombin time, and none in the inactive form (prothrombinogen) which in the adult constitutes about 75 per cent of the total prothrombin. This probably accounts for the finding that when prothrombin is measured by the two-stage method, its concentration in the blood of the newborn infant is only about 25 per cent. It is likely that the inactive prothrombin functions as a reserve, and is not immediately available. Recently it has been found that the adult dog requires only 0.5 gamma of vitamin K_1 per kilogram of body weight daily, whereas the growing pup needs over ten times as much, thus indicating that rapid growth greatly increases the organism's demand for vitamin K. Since vitamin K_1 is poorly absorbed, the amount available even when the diet is adequate may scarcely meet the needs of the fetus, and does not allow any storage. The baby is thus born without any appreciable supply of vitamin K and no reserve of inactive prothrombin. A temporary lack of vitamin K is therefore apt to cause a rapid and severe diminution of prothrombin.

89. THE RELATIONSHIP OF THE ANTIGENIC CHARACTERISTICS OF STREPTOCOCCI AND SPECIFIC ANTIBODY RESPONSES FOLLOWING STREPTOCOCCAL INFECTIONS

ROBERT W. QUINN, M.D., AND C. V. SEASTONE, M.D. (BY INVITATION), MADISON, WIS., AND ROBERT W. WEBER, M.D. (BY INVITATION), GREAT LAKES, ILL.

A correlation of clinical and antibody response in patients with beta hemolytic streptococcal infections with some of the biological characteristics of the homologous organism responsible for the patient's infection is presented in this study. There were 111 individuals and the corresponding number of strains of bacteria involved in this study. The patients were observed

clinically. Blood specimens were obtained *before* the infection in over half of the patients, during the acute phase in the remainder, and at appropriate intervals during convalescence. Antistreptolysin O, antistreptokinase, and antihyaluronidase determinations were made. The following characteristics were determined for each strain recovered from the patients: group and type, and streptolysin, streptokinase, hyaluronidase, and hyaluronic acid production.

No correlation of Lancefield group and type or biological characteristics of the infecting organism and clinical response could be established. Patients infected by apparently identical strains exhibited differences in clinical response, and those who had illnesses of similar severity and duration were often infected by organisms differing in the characteristics studied. Failure to show a rise in antibody to a given streptococcal antigen was observed, although the organism elaborated that antigen. Moreover, antibody rises were frequently observed in patients whose infecting organism could not be shown to give rise to the corresponding antigen. There was no consistent relationship between Lancefield type and the formation of streptokinase, hyaluronic acid, or hyaluronidase. A slight suppressive effect of treatment on the antibody response was observed, but it was minimal and by no means consistent. Five patients acquired rheumatic fever. There were no consistent differences in the biological characteristics between the strains which caused infection followed by rheumatic fever, and those which caused infections not followed by rheumatic fever. Four of the five rheumatic fever patients received an antibiotic during the streptococcal infection which preceded rheumatic fever; however, the amount which three of the five received would hardly be considered adequate dosage according to present-day practice.

90. ANTIBODY PRODUCTION AND TUBERCULIN SENSITIVITY IN INDIVIDUALS WITH A HISTORY OF RHEUMATIC FEVER

ROBERT W. QUINN, M.D., C. V. SEASTONE, M.D. (BY INVITATION), AND HELEN A. DICKIE, M.D. (BY INVITATION), MADISON, WIS.

It has been postulated by some workers that individuals who acquire rheumatic fever possess an altered capacity to react to antigenic stimuli, particularly those due to the group A hemolytic streptococcus. It is also conceivable that rheumatic individuals might also react in an abnormal manner to any antigenic stimulus. It is manifestly impossible in natural infections to obtain uniform antigenic stimulation, and it is extremely difficult to administer a single streptococcal antigen. Because of these difficulties, a standard antigen was administered to rheumatic and nonrheumatic subjects in the form of purified Type I pneumococcal polysaccharide followed by quantitative antibody nitrogen determinations. In addition, comparison of the capacity to develop bacterial allergy to tuberculin following immunization with BCG was made in similar groups of rheumatic and nonrheumatic persons.

The group described as rheumatic consisted of individuals with valvular heart disease due to rheumatic fever or a history of rheumatic fever or chorea. Each rheumatic group was compared with a group of nonrheumatic individuals of the same average age having no evidence of valvular heart disease and no history of rheumatic fever or chorea.

In the studies of antibody response, each subject received 0.05 mg. of Type I pneumococcal polysaccharide intradermally. Blood samples were obtained from each individual immediately before and one month after immunization. The method of Heidelberger and associates for quantitative antibody determination was followed.

Tuberculin negative (1.0 mg.) rheumatic and nonrheumatic individuals were employed to study tuberculin sensitivity following BCG. BCG vaccine concentration was 40 to 50 mg. per milliliter; the same lot of old tuberculin was used throughout.

It is apparent from the results that the rheumatic individuals in this study (19) showed no consistent tendency to produce larger amounts of pneumococcal polysaccharide antibody than nonrheumatic (22), or a greater degree of tuberculin sensitivity (12 rheumatic) than did nonrheumatic persons (20). It is felt that the basic idea that rheumatic fever is related to some sort of hypersensitivity to beta hemolytic streptococcal infection is not invalidated by these results.

91. SENSITIVITY TO PETROLEUM INCLUDING ITS DERIVATIVES AND ANTECEDENTS

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

Recognition of the clinical picture resulting from petroleum sensitivity has been delayed because of the ubiquitous distribution of petroleum and wood products.

Forty cases, each exhibiting evidence of sensitivity to certain derivatives or antecedents of petroleum, have been studied during the past two years. The extreme case of a physician's wife and former cosmetics saleswoman, aged 41 years, initiating this study and exhibiting more severe reactions to a wider range of specific exposures than any other subject will be reported, for knowledge of such a case aids in the recognition of less severe and more common instances.

The patient had been subject to contact dermatitis, perennial nasal allergy, headaches, intractable coughing and asthma, severe fatigue, intermittent generalized edema, and unexplained episodes of loss of consciousness. She was also sensitive to house dust, several foods, mercury, and natural opiates. There is clinical evidence of sensitivity to the following petroleum and pine products:

Contactants:
- Nail polish
- Lipsticks
- Certain dyes in clothing and soap
- Certain face powders and creams
- Petroleum ointment bases and jellies
- Lysol
- Synthetic alcohol
- Nylon and certain other plastics
- Pine oil as used in baths

Ingestants:
- Aspirin
- Phenacetin
- Sulfonamides
- Benzedrine
- Certain synthetic vitamins
- Certified food colors (dyes) in foods, soft and alcoholic beverages
- Wood ethyl alcohol

Inhalants:
- Automotive and fuel oil stove exhausts
- Gas stove, gas refrigerator, and oil furnace exhausts
- Gasoline and refinery fumes
- Many perfumes and colognes
- Toilet deodorants
- Benzedrine
- Paint and turpentine fumes
- Knotty pine interiors and cedar chests
- Pine forests (outdoor and indoor—Christmas trees, and decorations)
- Burning of pine in fireplace

Injectants:
- Phenol (as preservative)
- Benadryl
- Methodon
- Meperidine

She reacted positively to a U.S. certified food dye by trial ingestion and by patch tests (1/100 recommended strength) and to ingestion of wood ethyl alcohol. Several others have reacted positively to ethyl alcohol derived from ethylene gas or wood, whereas controls with negative petroleum histories

failed to react. The clinical problem presented by the aspirin-sensitive patient is apparently a part of this clinical picture of petroleum and pine sensitivity. Avoidance of exposures, though difficult, is reasonably effective therapy.

92. THE ALTERNATION OF THE SYMPTOMS OF ALLERGY AND THOSE OF ALCOHOLISM AND CERTAIN MENTAL DISTURBANCES

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

The alternation of infantile eczema and respiratory allergic symptoms has long been recognized, the dermatitis clearing coincidentally with or being followed by the onset of rhinitis or asthma or both. Adults subject to both dermal and respiratory allergy more frequently exhibit primarily an accentuation of either eczema or asthma than both simultaneously.

A similar alternation of bronchial asthma and alcoholism was pointed out by Hare in 1913, and several such instances have been observed. One alcoholic had hay fever only prior to and following a ten-year period of daily compulsive drinking. Another steady drinker developed initial asthma coincident with cessation of drinking in preparation for an insurance examination. Since subsequently becoming a periodic drinker, he invariably develops asthma beginning two hours after taking the last drink, which persists for the following three to ten days providing he continues to abstain.

Salter, in 1882, pointed out the temporary effectiveness of increasing doses of alcoholic beverages in relieving asthma. In my experience 10 per cent of a group of 150 alcoholics reported relief of their asthma as a result of the continuous ingestion of their favorite alcoholic drink. An additional significant percentage observed that their rhinitis or headaches were not troublesome as long as they continued to drink regularly.

Alternation of asthma, hay fever, eczema, and migraine with insanity was pointed out by Savage in 1882, and similar cases of alternating asthma and mental disturbances have been observed subsequently. The presence of either asthma and hay fever *or* depression has not only been noted in several patients, but in one instance the blind intubation of an allergenic food has been followed on repeated occasions by transitory rhinitis, coughing, or urticaria which subsided coincidentally with the onset of progressive hyperkinesis and psychotic behavior persisting for two or three days. With improvement the subject first became reoriented and then developed a recurrence of rhinitis and coughing. Less-advanced instances of the sequence of coughing or rhinitis followed by mental reactions have been observed on numerous occasions following the test ingestion of allergenic foods.

93. THE NATURE OF THE CLOTTING DEFECT IN A CASE OF AMNIOTIC FLUID EMBOLISM

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(INTRODUCED BY AUSTIN S. WEISBERGER, M.D.)

Pulmonary embolism by amniotic fluid is an occasional cause of maternal death. Generalized hemorrhagic phenomena may be observed in patients with this disorder who survive an initial phase of shock. Weiner, Reid, and Roby have demonstrated that amniotic fluid is thromboplastic, initiating the conversion of prothrombin to thrombin in the presence of calcium. They therefore suggested that the bleeding phenomena in patients with amniotic fluid

embolism might be due to defibrination of the maternal blood associated with intravascular clotting.

A 30-year-old white woman died eleven and one-half hours post partum after a prolonged period of shock. At autopsy, amniotic fluid emboli were observed. Three hours after the onset of shock, signs of a generalized hemorrhagic state became evident. The patient's blood appeared to be incoagulable. This clotting defect was associated with hypofibrinogenemia, excessive plasma thrombin-inhibitory activity, and slight thrombocytopenia. Similar defects have been described in dogs injected with sufficient homologous thromboplastin to cause partial or complete defibrination. In addition, the patient's plasma was excessively fibrinolytic. The data obtained lend support to the concept that the hemorrhagic state in patients with amniotic fluid embolism may be related to the thromboplastic activity of the amniotic fluid.

94. PRE- AND POSTOPERATIVE TORSION BALLISTOCARDIOGRAMS IN SURGICALLY AMENABLE CARDIOVASCULAR DISEASES

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(INTRODUCED BY WILLIAM C. MENNINGER, M.D.)

The ballistocardiogram was analyzed before and after operation in more than one hundred cases of surgically amenable cardiovascular diseases. Definite disease patterns could be established in patent ductus arteriosus, coaretation, constrictive pericarditis, and mitral valve disease with striking change toward normal after operation. The association of specific BCG patterns with a degree of mitral regurgitation or stenosis was possible.

The patterns in the BCG were correlated with the abnormal hemodynamics by right heart catheterization and by direct pressures and samples obtained by needle during surgery. A torsion ballistocardiograph was used which permits an accentuation of the pathologic deviation by positioning the patient relative to the center of rotation.

95. ARTERIOPHONOGRAPHY: A NEW MEASURE OF CARDIOVASCULAR FUNCTION

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(BY INVITATION), CHICAGO, ILL.

A simple method has been devised which provides qualitative information concerning (a) blood flow through extremities, (b) elasticity of the central aortic and arterial tree, and (c) evidence for changes in stroke output. To obtain these data blood pressure is measured by the usual sphygmomanometric technique. The sounds produced at the brachial, radial, or popliteal artery and an electrocardiogram are recorded simultaneously. The intensity of the sound produced is related to the flow of blood through the extremities. This is demonstrated by the increased sounds heard during reactive hyperemia and the decreased sounds after occlusion of the peripheral vessels. Following the R wave, a lag of about 0.2 second transpires before the Korotkoff sound is recorded at the brachial artery. A plot of the duration of the lag against the pressure in the cuff makes it possible to construct a pressure-pulse contour. The lag is a function of the stroke output and of the elasticity characteristic of the central arterial tree. One hundred determinations at the brachial arteries of 20 subjects reveal that the lag is about 0.18 second at the onset of systole, and 0.26 second at peak of systole. The lag is unaffected by

local changes in blood flow, but is considerably decreased by a rise in cardiac output, and the slope of the curve is increased. The possible clinical utilization of arteriophonography in this connection will be discussed.

96. VIBRATION ANALYSIS OF HEART SOUNDS AND MURMURS

SIMON RODBARD, M.D., CHICAGO, ILL.

Heart sounds and murmurs have been studied with the aid of a Kay vibration analyzer suitably adjusted. This instrument provides a continuous display of the frequencies and amplitudes of sound versus time. The normal first heart sound at the apex is shown as a series of about 20 transients, each about 3 milliseconds in duration. The frequency pattern of these transient sounds shows energy components, adequate for recording, up to about 1,000 cycles per second. An envelope around the first heart sound usually shows three to four main periods, each about 10 to 15 transient periods, usually with somewhat higher frequency components than the first sound; the envelope shows two or three main periods. Murmurs are also recorded as transients. Graphic analyses of Korotkoff sounds and of some heart sounds show striking similarities to those produced in artificial circulation models undergoing flutter. The vibration analyzer makes it possible to display certain characteristics of heart and vessel sounds which cannot be illustrated by conventional galvanometric or oscilloscopic techniques. Its clinical utility is under investigation.

97. THE EFFECTS OF NITROGEN MUSTARDS AND TRIETHYLENE MELAMINE IN DISSEMINATED LUPUS ERYTHEMATOSUS

ROBERT J. ROHN, M.D., AND WILLIAM H. BOND, M.D., INDIANAPOLIS, IND.

(INTRODUCED BY PAUL J. FOUTS, M.D.)

The nitrogen mustards have long been employed in the treatment of various hematologic disorders. More recently they have been found to be effective in the reversal of sensitization disorders, and in the treatment of some of the nephroses and rheumatoid arthritis. No one has, however, employed nitrogen mustards in disseminated lupus erythematosus except as adjunctive therapy with corticosteroids in the treatment of the nephropathies associated with disseminated lupus.

Five patients with acute disseminated lupus erythematosus proved by clinical findings, significant numbers of L.E. cells in bone marrow and peripheral blood, and biopsy of skin lesions were selected for a trial with nitrogen mustards (HN_2) and triethylene melamine (TEM) therapy. Four patients were females and one was a male. In one patient corticosteroids were contraindicated because of complicating active pulmonary tuberculosis. In another patient corticosteroids had proved too toxic at effective maintenance dosage levels.

Laboratory studies included quantitation of bone marrow L.E. cells, hemograms, sedimentation rates, total proteins and A-G ratios, electrophoretic patterns, and excretion of 17-ketosteroids, 11-oxysteroids, uric acid, and creatinine. The effect of HN_2 and TEM upon these values is presented, and in some patients this is contrasted with corticosteroid effects.

In two patients HN_2 and TEM effected repeated good remissions, although such remissions were not permanent. In one patient fair remission was obtained with HN_2 and TEM after effective maintenance corticosteroid ther-

apy proved too toxic. In two patients HN_2 proved ineffective in securing remission, although in one of these corticosteroids proved equally ineffective. Illustrative slides will be shown.

Our results would suggest that the effect of HN_2 and TEM is exclusive of pituitary adrenal stimulation, and further trial with these agents would be indicated in those patients in whom corticosteroids are contraindicated or proved ineffective.

98. PARTIALLY IRREVERSIBLE LEFT VENTRICULAR ENLARGEMENT ASSOCIATED WITH PATENT DUCTUS ARTERIOSUS

FRANCIS F. ROSENBAUM, M.D., MILWAUKEE, WIS.

Patent ductus arteriosus is associated only occasionally with pronounced cardiac enlargement. It has been our experience that the slight enlargement commonly accompanying this lesion clears promptly after successful surgical closure; reversion to normal has been observed also in two children with rather marked enlargement, although the reduction in cardiac size required several months. Two young adult males with very marked cardiac enlargement have been observed for three and one-half years following successful operative closure of a patent ductus arteriosus. Gradual reduction in cardiac size has occurred, but a level was reached beyond which no further decrease occurred; in fact, in one patient radiographic observations suggest some retrogression. Orthodiagraphic studies in one patient disclosed that the frontal area was +94 per cent and the transverse diameter +44 per cent above normal values prior to operation and +15 per cent and +17 per cent, respectively, forty months postoperatively. In the second patient the frontal area was +43 per cent and the transverse diameter +28 per cent prior to operation and +29 per cent and +16 per cent, respectively, forty-one months postoperatively. Electrocardiograms in both patients displayed striking evidence of left ventricular hypertrophy preoperatively and, although there has been considerable clearing, distinct abnormalities persist in the tracings. Excellent clinical responses have occurred in both patients, and each is performing normal work.

These cases resemble the single case reported by Dry, Harrington, and Edwards of a patient who died four years after successful ligation of a patent ductus, although in that instance reduction in the size of the heart was not observed at any time postoperatively. These patients are in part a clinical counterpart of the experiment of Eyster in which transient aortic overloading and left ventricular dilatation resulted in ultimate left ventricular hypertrophy, even though the aortic narrowing was released early in the experiment. The observations in these patients point up the fact that there are important factors in the production of ventricular hypertrophy which are still poorly understood, for, despite restoration of a normal circulation, ventricular enlargement has been partially irreversible. This study suggests that surgical closure should be carried out in patent ductus arteriosus as early as the diagnosis is made with certainty, at least before the age of 10 years.

99. NEW CYTODIAGNOSTIC METHODS IN GASTROINTESTINAL MALIGNANCY

CYRUS E. RUBIN, M.D., CHICAGO, ILL.

(INTRODUCED BY JOSEPH B. KIRSNER, M.D.)

Disappointing reports have appeared concerning the diagnostic accuracy of gastrointestinal exfoliative cytology. The present study seeks answers to two

questions. How reliable and useful is cytodiagnosis clinically? Can improvements and simplifications of technique overcome present limitations? One hundred seventy-five cases were studied; 140 have been proved benign or malignant by pathologic examination or subsequent clinical course. The cytologic method demonstrated greater diagnostic accuracy than x-ray or endoscopy in this series. No false cytologic diagnoses of cancer have been made to date. Poor results reported by others are partly attributable to cell digestion and aspirate contamination with food and detritus. Careful patient preparation and new collection methods minimize these difficulties. Lavage obtains excellent cytologic material from the esophagus. Buffered papain added after aspiration insures even cellular distribution by eliminating interfering mucus. "Saline" or "papain lavage" and the "gastric abrasive balloon," described by others, produce good gastric cells; however, comparative study of these methods reveals limitations in all. The "antral abrasive balloon" developed in this laboratory has proved useful. It abrades the antrum, an area of cancer predilection often inaccessible to other methods. "Chymotrypsin lavage," a promising new method developed in collaboration with Dr. E. P. Benditt, releases the well-protected cells contained in the mucous barrier covering the gastric mucosa. Secretagogues and other technical modifications have been employed in "duodenal drainage" in an effort to diagnose pancreatic, biliary tract, and hepatic cancer. Colonic enema is most useful in distal lesions. "Magnetic positioned enemas" and other techniques are being explored in an effort to reach distal lesions. The procedure of "total surface suction" aspirating cells from the whole surface of lesions also has been devised. This technique has been a useful adjunct to conventional diagnostic methods. A modified Papanicolaou stain is helpful. New improved methods in gastrointestinal exfoliative cytology make this form of diagnosis more widely applicable.

100. CRANIOSPINAL TRANSCAPILLARY SEEPAGE: THE MECHANISM FOR THE EFFECTS OF CHANGES IN THE OSMOTIC PRESSURE OF THE BLOOD ON THE CEREBROSPINAL FLUID PRESSURE

HENRY W. RYDER, M.D., FRANK F. ESPEY, M.D., FARISS D. KIMBELL, M.D., LT.(JG) ERNEST J. PENKA, (MC), U.S.N., ADOLF ROSENAUER, M.D., BORIS PODOLSKY, PH.D., JOSEPH P. EVANS, M.D., DORIS LAMB, B.S., EDYTHE BARNES, B.A., JOAN JACKSON, M.S., AND WILLIAM BROSENE, B.S., CINCINNATI, OHIO

When anyone drinks water, the pressure of the cerebrospinal fluid gradually rises, reaching a peak in about twenty minutes. The greater the initial pressure, the higher is the rise. Opposite effects are seen following the administration of hypertonic glucose. A basic question is whether the mechanism of these responses is the same for all men, or, when the pressure is high, whether there is either an exaggeration of a normal process or even a pathologic reaction. The answer is approached by determining whether both the rise in pressure and the disproportionality may be explained by a mechanism common to all men.

The first step is to determine the net amount of water that shifts across the craniospinal capillary walls as a result of changing the osmotic pressure of the blood. Whenever the volume of fluid in the craniospinal fluid spaces is changed, the effect on the fluid pressure depends on the initial craniospinal blood volume; the lesser the volume, the greater is the pressure response. A particular change in fluid pressure may be expressed in terms of a specific

change in blood volume in milliliters, and this is the negative of the change in fluid volume, when the cerebral blood flow is constant. By determining the craniospinal pressure-volume curve for any man, and his pressure responses to the ingestion of water and the injection of glucose, the pressure response may be expressed in terms of the net shift of water across the craniospinal capillary walls that causes this change in pressure. Analysis of the data shows that the mean fluid shift is plus 3.4 milliliters per liter of water ingested, and minus 3.2 milliliters per 50 ml. of 50 per cent glucose injected. The shift is independent of the initial fluid pressure or the condition from which the patient was suffering.

Therefore, we may infer that the Starling equilibrium controls the net craniospinal transcapillary seepage.

101. THE EFFECT OF ANTI-TUBERCULOUS THERAPY ON SERUM MUCOPROTEINS AND GAMMA GLOBULIN

FENTON SCHAFFNER, M.D., AND GEORGE C. TURNER, M.D., CHICAGO, ILL.

(INTRODUCED BY HANS POPPER, M.D.)

Serum gamma globulins, mucoproteins, and polysaccharides were determined at regular intervals in 45 patients with far-advanced pulmonary tuberculosis. The findings were correlated with the results of antituberculous treatment instituted. This included isoniazid, streptomycin-PAS, and combinations of all three. The concentration of the serum components was considered a nonspecific host reaction to inflammation or tissue breakdown. These were determined by simple laboratory procedures such as turbidimetric estimation in the case of gamma globulins, protein determination for mucoproteins, and the diphenylamine reaction for polysaccharides. The results were correlated with other nonspecific reactions such as the sedimentation rate and white count, as well as the clinical picture. Only the patients studied for longer than six months were considered.

The serum gamma globulins were abnormally high in more than four-fifths of the cases initially. Subsequently, in about one-half the concentration decreased and in one-half it increased during all types of therapy. There was no clear correlation with clinical features or other laboratory findings except that an increase in gamma globulin was frequently accompanied by an increase in the sedimentation rate. Elevation of mucoproteins above 10 mg. per 100 ml. was a poor prognostic sign, and was always associated with elevated gamma globulins. In two-thirds of the cases the mucoproteins were above normal (more than 4.0 mg. per 100 ml.). On all forms of therapy one-half of these dropped to normal. The polysaccharide values consistently dropped on all forms of therapy. If the ratio of polysaccharides to mucoproteins decreased markedly, clinical improvement failed to develop. Of the methods tested, mucoprotein values and determinations best mirrored the prognosis. Although symptomatic improvement seemed to occur earlier in the groups with isoniazid, no differences in mucoproteins and gamma globulins between these groups and the one on streptomycin-PAS therapy were noted.

102. CALCINOSIS UNIVERSALIS

A CASE WITH LONG-TERM TREATMENT WITH ACTH AND CORTISONE

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A 30-year-old white woman first became ill in 1938 with an acute febrile illness accompanied by pain and swelling of the extremities and tender sub-

cutaneous nodules which broke down discharging a chalky material. All skeletal muscles became woody and firm, and the patient was finally completely bedridden.

Within six months spontaneous improvement set in so that she was able to return to school. In 1941 there was a recurrence which again incapacitated the patient. From 1947 to 1951, when the patient was first seen by us, her condition was stationary.

The patient presented a remarkable picture. The gluteal muscles and those of the lower extremities were of stony hardness. The thighs were tightly adducted. There was no motion at hips or knees and very slight motion at the ankles. A pitting edema was present from the knees down. The upper extremities were atrophied, but there was only slight limitation of function. Irregular calcified nodules could be felt in the axillas, in the gluteal regions, and on the posterior aspect of the left thigh. Otherwise the examination was normal.

Routine laboratory examinations, blood calcium, phosphorus, and phosphatase were normal. On a 500 mg. calcium intake urinary excretion was 50 to 60 mg. A muscle biopsy from the arm was reported as dermatomyositis.

While hospitalized, the patient was given 100 mg. of ACTH intramuscularly daily for a period of a month. Urinary calcium excretion rose to a level of around 150 mg. per day. She felt more limber. There was definite softening of the thigh muscles.

Because of these encouraging findings, it was decided to place the patient on long-term treatment with oral cortisone. She was given 75 to 100 mg. per day for a period of eight months. The high level of urinary calcium excretion continued, but clinically the patient remained unchanged. Comparison of x-rays before and after treatment showed no significant change. The slight increase in goniometric measurements we attribute to physiotherapy rather than the medication.

103. OBSERVATIONS IN MAN ON CEREBROSPINAL FLUID PRESSURE, pH AND ON RESPIRATION DURING EXPERIMENTAL METABOLIC ALKALOSIS

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(INTRODUCED BY B. K. WISEMAN, M.D.)

The increase in cerebral blood flow (CBF) after 5 per cent CO_2 inhalation is associated with hyperpnea, abrupt increase in cerebrospinal fluid (CSF) pressure, and decrease in CSFpH. The comparable increase in CBF following intravenous infusion of 3 per cent $NaHCO_3$ is not accompanied by clinically observable change in breathing. Observations in man have been made on changes in measured respiration, and in CSFpH and pressure during experimentally produced metabolic alkalosis.

Alkalosis was induced by intravenous infusion of 1,000 ml. of 3 per cent $NaHCO_3$ over thirty to fifty minutes. In six healthy young volunteers breathing room air, respiration was recorded by spirometry, and arterial blood samples were obtained for pH, CO_2 tension, and CO_2 content. In four essentially physically normal hospital patients, in whom lumbar puncture was contemplated, CSFpH and pressure were measured before and during bicarbonate infusion.

Metabolic alkalosis, as produced by $NaHCO_3$, does not change either the rate or depth of breathing nor the total minute respiratory volume. Although arterial blood became more alkaline (7.38 to 7.52), CSFpH became more acid (7.40 to 7.25). CSF pressure gradually but only slightly increased (145 to 166

mm. H_2O) in contrast to abrupt high increases in pressure often reaching over 300 mm. H_2O after CO_2 inhalation.

In summary, metabolic alkalosis per se is not associated with decreased breathing. Although both inhalation of 5 per cent CO_2 and infusion of $NaHCO_3$ increase CBF comparably, the bicarbonate causes little change in CSF pressure. The change in CSFpH after bicarbonate noted in 1926 by Gesell in dogs is confirmed in man.

104. THE MECHANICAL FRAGILITY OF ERYTHROCYTES FROM LEUKEMIC PATIENTS

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(INTRODUCED BY OVID O. MEYER, M.D.)

As early as 1935, Jaffe suggested, on the basis of microscopy, that an increased rate of red cell destruction might be contributory to the anemia seen in leukemic patients. More recently, Ross and Berlin independently reported red cell survival studies and fecal urobilinogen data demonstrating hemolysis as a cause of the anemia of leukemia.

This is a report of data showing that the erythrocytes from leukemic patients have an increased mechanical fragility in vitro. Measurements conducted on 43 leukemic bloods show that the average mechanical fragility was 6.5 per cent, while the average for the 43 nonleukemic, nonanemic control bloods was 4.4 per cent. Statistical analysis of these data demonstrates that the difference in these average values is highly significant. Hemolytic anemia was diagnosed clinically in only one of the 43 patients. The average mechanical fragility for the 12 bloods of acute leukemia included in this series of 43 leukemic bloods was 7 per cent.

The finding of an increased mechanical fragility of the erythrocytes from leukemic patients is consistent with previous studies of a different nature reporting an increased rate of hemolysis as commonly contributory to the anemia of leukemic patients.

105. DISTURBANCE OF CATALASE METABOLISM IN EXPERIMENTAL PORPHYRIA

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(INTRODUCED BY C. J. WATSON, M.D., PH.D.)

Two fundamentally different forms of experimental porphyria have recently been produced in rabbits in this laboratory. One, caused by phenylhydrazine, lead, and ultraviolet radiation, has great similarity with the human congenital photosensitive type (porphyria erythropoietica). The excessive porphyrin formation in both takes place in the bone marrow as a result of a disturbed hemoglobin synthesis. The other form, which has some features of the human intermittent acute type (porphyria hepatica), is produced by Sedormid, and is believed to be due to a disturbance of liver cell metabolism.

In normal animals and under a variety of pathologic conditions, the liver contains a relatively high concentration of the iron-porphyrin-enzyme catalase. In Sedormid porphyria, the liver catalase concentration is significantly decreased. Oral Sedormid administration to rabbits for only two to three days already results in a moderate decrease of catalase concentration and a simultaneous increase in porphyrin concentration in the liver. If Sedormid treatment is continued for several days, until the urinary uroporphyrin excretion has

reached values in excess of 10,000 μg per day, the liver catalase concentration decreases to one-twentieth or less of normal values. At the same time the catalase concentration of other organs, e.g., the erythrocytes and the kidneys, is uninfluenced, and there is no disturbance of hemoglobin metabolism in such animals.

In experimental erythropoietic porphyria, on the other hand, where the abnormal porphyrins are formed in the immature erythrocytes of the bone marrow, no change in catalase metabolism is found in liver, erythrocytes, or kidneys. Starvation, carbon tetrachloride poisoning, and the addition of Sedormid to liver homogenate, in vitro, do not influence the catalase activity in the rabbit liver.

These data suggest the possibility that the excessive hepatic porphyrin formation in Sedormid poisoning is the result of a disturbed catalase metabolism in the liver cells, analogous to the disturbance of hemoglobin synthesis by lead in the developing red cell. Studies of the hepatic catalase concentration in human porphyria are in progress and will be discussed briefly.

106. FURTHER EXPERIENCE WITH SPECIFIC ANTI-SICKLE-CELL ANEMIA RABBIT SERUM IN THE DIAGNOSIS OF SICKLE-CELL ANEMIA

ROSE G. SCHNEIDER, PH.D., AND WILLIAM C. LEVIN, M.D., GALVESTON, TEXAS

We have previously reported that injections of sickle-cell anemia erythrocytes into rabbits resulted in the production of antiserum which specifically agglutinated sickle-cell anemia erythrocytes, but not those of sickle-cell trait. This experience has been enlarged so that at present, erythrocytes from 27 patients with sickle-cell anemia have been agglutinated by the serum, while there was no agglutination of erythrocytes from 57 patients with sickle-cell trait nor of 224 normal individuals. Three other patients with sickle-cell trait reacted positively on one occasion and negatively on others. Three particularly interesting cases were encountered, which illustrate the need for reliable diagnostic procedures to differentiate between the various types of sickle-cell disease.

F. T., a 9-year-old Negro boy, was diagnosed clinically as sickle-cell anemia; however, his erythrocytes were not agglutinated by the serum. Clinical reevaluation one year later revealed no anemia and only minimal sickling. The diagnosis of rheumatic heart disease was evident.

J. C., a 65-year-old Negro man, had been well until three years before admission, when chronic leg ulcers developed. His erythrocytes were agglutinated by the serum and hematologic study indicated an unequivocal diagnosis of sickle-cell anemia.

The most interesting case is that of I. C. G., a 19-year-old Negro man, referred to us with a diagnosis of sickle-cell anemia. Clinically, the patient presented an indolent leg ulcer, a moderate normochromic anemia, slight reticulocytosis, and marked sickling. His erythrocytes were not agglutinated by the antiserum. Subsequently, the leg ulcer healed and the severity of the anemia decreased. Electrophoretic studies of the hemoglobin show three peaks, produced by normal, sickling, and the third type of hemoglobin, indicating that this patient does not have true sickle-cell anemia.

These data support the thesis that the antiserum described is a useful diagnostic aid in differentiating sickle-cell anemia from sickle-cell trait associated with unrelated anemia. To date, erythrocytes from no patients with proved sickle-cell anemia have failed to be agglutinated by the antiserum; however, instances of alternately positive and negative reactions were encountered in three patients with sickle-cell trait. These inconsistencies require further study.

107. STUDIES IN MAN ON ENDOTHELIAL PERMEABILITY TO RADIOACTIVE IODINE-TAGGED ALBUMIN INJECTED INTRAVENOUSLY OR INTRAPERITONEALLY

JAMES A. SCHOENBERGER, M.D. (BY INVITATION), GEORGE KROLL, M.D. (BY INVITATION), ARTHUR SAKAMOTO, M.D. (BY INVITATION), AND ROBERT M. KARK, M.R.C.P., F.A.C.P., CHICAGO, ILL.

Radioactive iodine-tagged human albumin was used to study endothelial permeability in various disease states associated with ascites and edema. The material was injected in tracer doses either intravenously or intraperitoneally, and its concentration at different sites was measured at frequent intervals after injection for as long as fourteen days. The data were represented graphically, plotting concentration of radioactivity against time. From the curves it was possible to calculate, among other results, the total exchangeable albumin pool, the rate of daily turnover, and the half-life of the albumin molecule.

In order to test the previously advanced hypothesis that malnutrition was associated with loss of endothelial semipermeability, these experiments were performed at intervals in some individuals to observe the effects of a high-protein rehabilitating diet on permeability.

The studies disclosed a dynamic equilibrium between albumin in intravascular and extravascular sites. Mathematical analysis of the curves yielded quantitative estimates of the rate of exchange of albumin across the peritoneal membrane and also out of the vascular endothelium. For example, in one patient studied at two different times after intravenous injection of I^{131} albumin, it was found that initially 0.410 Gm. of albumin entered the peritoneal cavity per hour. A similar amount left the cavity, since the patient was in a steady state. Three months later analysis showed that 0.399 Gm. of albumin crossed the membrane per hour. Initially, 4.4 per cent of the tagged albumin moved out of the patient's plasma per hour, while on the second occasion, 4.8 per cent per hour left the plasma. The clinical implications of these studies will be discussed.

108. FURTHER OBSERVATIONS ON THE CONTROL OF HYPERTENSION BY HYPHEX

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Two hundred patients suffering from arterial hypertension were treated continuously by oral hexamethonium chloride and 1-hydrazinophthalazine (Hyphex) for periods up to thirteen months. Control was begun in the hospital by published method (*Arch. Int. Med.* **89**: 523, 1952). All cases demonstrated secondary pathologic changes in the heart, brain, or kidneys. The blood pressures of all were markedly reduced.

Of 14 with congestive cardiac failure, 12 remain completely relieved without digitalis, diuretics, or restriction of salt. Failure did not disappear in 2 with arteriosclerotic heart disease.

Of 21 with nitrogen retention of not more than twice normal levels, 8 no longer exhibit it, and 13 are unchanged. Of 10 with serious nitrogen retention, one recovered and 3 are considerably improved.

Of 26 with old or recent cerebral accidents, one developed a subsequent minor episode while on treatment. Of those without previous attacks, 2 developed cerebral thrombosis on treatment, and 3 others developed it during induction of control. Four have recovered.

Of 5 with angina pectoris, one is improved and 4 are free from attacks. Old coronary occlusion had occurred in many, but only one has had a subsequent episode.

The malignant stage has regressed in all of 51 cases with or without serious nitrogen retention. One patient died of the effects of hypertension while on treatment. Of 9 from whom the drugs were withdrawn, all died of the effects of hypertension shortly thereafter.

Eight deaths occurred on treatment: 2 from pneumonia, 3 with uremia from hexamethonium poisoning, one from coronary occlusion, and 2 with uremia from unknown causes while normotensive.

No drug idiosynerasies have been observed after induction of control. No tolerance has developed. One patient remains normotensive after cessation of therapy; 12 have been able to reduce dosage 40 per cent or more after six months of treatment and maintain normotension. Undesirable effects of autonomic blockade have diminished or disappeared in every case treated for more than three months. Hydrazinophthalazine administered for more than three years has caused no late toxic effects. Hyphex appears to be as safe as any regimen which is effective for control of hypertension.

109. THE COMPARATIVE ASSAY OF ACTH IN MAN

ALBERT SEGALOFF, M.D., RALPH A. CARABASI, M.D. (BY INVITATION), AND B. N. HORWITT, PH.D. (BY INVITATION), NEW ORLEANS, LA.

Short-term assays of ACTH potency in man, particularly involving measures such as eosinopenia and uric acid-creatinine ratio changes are difficult to assess and are affected by various psychic and other influences. An attempt was, therefore, made to employ an assay procedure which eliminates some of these objections. A longer term of therapy, namely, four days, was employed, and urinary excretion of 17-ketosteroid and formaldehydogenic corticoids were measured on the third and fourth days of therapy. Aqueous preparations of ACTH were compared by administering small volumes with a continuous injection apparatus subcutaneously, intramuscularly, or intravenously. Gel preparations were given intramuscularly once daily. Using gels of various types and a two-dose assay design in the same patients, it was possible to show that there is a marked disparity between the labeled potencies of the materials as determined by Sayers' animal assay and the efficacy in man by this assay method. Various aqueous or lyophilized preparations when compared with each other differ in their apparent potency with the route of administration. While some preparations were most effective when given intravenously, one preparation, which was aqueous, gave the greatest response when given subcutaneously.

110. STUDIES ON THE ACTION OF INTRAVENOUSLY ADMINISTERED TRYPSIN

SOL SHERRY, M.D., WALTER TROLL, PH.D., AND LEONARD GOTTESMAN, M.D., CINCINNATI, OHIO

(INTRODUCED BY MORTON HAMBURGER, M.D.)

The demonstration by Innerfield and associates that crystalline trypsin when slowly infused intravenously can be safely administered to man in fairly large dosage has stimulated studies on its therapeutic potentialities as a fibrinolytic agent.

In exploring the in vivo action of trypsin, biochemical studies have been made in conjunction with the intravenous infusions of this enzyme into normal dogs. The amount of trypsin varied from 150 to 500 mg. per animal, and was given at a rate of 40 to 100 mg. per hour.

Tryptic activity was demonstrable in the blood and plasma during the infusion, and for considerable periods thereafter. Some tryptic activity appeared to circulate with the red cell.

The initial level of tryptic activity was determined by the rate of trypsin infusion, and the trypsin inhibition of the plasma. The presence of free tryptic activity did not depend, as generally believed, on exceeding the plasma inhibition. The trypsin inhibitors either do not combine completely with the infused trypsin, or appear to compete with substrates for the enzyme. In this way they determine the per cent of the total enzyme available for attack on substrates. The maximum levels of tryptic activity achieved represented about 1 to 3 per cent of the total enzyme infused, regardless of dosage. In in vitro experiments, we could not exceed this small percentage of free tryptic activity even by the addition of 2 mg. of trypsin per milliliter of plasma (200 mg. per cent).

Other physiologic mechanisms for the disappearance of infused trypsin from the plasma were present, since the level of free tryptic activity slowly fell, in spite of the continuous infusion of constant amounts of the enzyme. Urinary excretion of small amounts of tryptic activity occurred, accounting, however, for little of the tryptic activity lost.

Clottable fibrinogen and prothrombin rapidly disappeared during the trypsin infusion. The blood was rendered incoagulable, and the erythrocyte sedimentation rate fell to zero. The plasma NPN did not appear to be significantly elevated. In some of the animals, a drop in the serum proteins was observed. The effects described were of a transient nature, and restoration to normal values was completed within twenty-four to forty-eight hours.

111. EVALUATION OF THE RADIOCARDIOGRAM IN HEART FAILURE

REGINALD A. SHIPLEY, M.D., RICHARD E. CLARK, B.S. (BY INVITATION), AND DANIEL LIEBOWITZ, M.D. (BY INVITATION), CLEVELAND, OHIO

Prinzmetal, by injecting 100 μc of radiosodium intravenously, was able to obtain a graphical record of the rise and fall of radioactivity over the precordium during the passage of the tracer material through the chambers of the heart. Distortions in contour of the curves were noted in the presence of cardiac disease.

In the present experiments, it has been possible to obtain satisfactory curves with I^{131} iodinated albumin. A scintillation counter was used for detection. Doses as low as 3 to 5 μc could be used. Twenty-three normal subjects and eleven cardiac patients in failure have been studied. For the most part, the cardiac patients had been digitalized and had already improved in great measure during hospitalization. The tracings were analyzed with the intent of deriving indices of cardiac output which would permit quantitative comparison of the normal and decompensated groups.

A simple index of cardiac output is rate of flow. Measurement of the time interval between the two peaks of activity which represent the maxima of the right and left heart gave normal values for lung time of from 3.5 to 6.7 seconds (mean 5.1). Cardiac patients ranged from 6.5 to 21.0 (mean 11.1). When correction was made for the overlap of the second peak by the extrapolated descending limb of the first, lung time became 4.5 to 7.7 seconds in normal subjects (mean 5.8) and 8.0 to 26.0 (mean 12.5) in decompensated patients.

Another index of cardiac output was derived by the principle of time-dilution of an injected test substance. The formula used was:

$$\text{Flow index} = \frac{\text{Height of tracing after mixing} \times \text{blood volume}}{\text{Area under total curve}}$$

Values so obtained for normal subjects ranged from 7.3 to 13.5 (mean 9.9) and for cardiac patients 2.4 to 8.1 (mean 4.8). Half-time for the declining limb of the second peak of the curve when extrapolated exponentially gave a comparably favorable differentiation of the two groups (normal subjects 2.0 to 5.8 sec., mean 3.8; cardiac patients 4.3 to 60.0 sec., mean 15.4).

The proportional magnitude of area under the right and left components of the tracing showed a slight correlation with the relative size of the comparable ventricle.

112. THE EFFECT OF SMOKING ON THE CIRCULATION AND SKIN TEMPERATURES OF NORMAL MEN

David L. Simon, M.D. (By Invitation), Arnold Iglauer, M.D., and Mary Jane Tompkins (By Invitation), Cincinnati, Ohio

The effect of smoking on cardiac output was measured by the critically damped high frequency ballistocardiograph. Thirty-two studies of cardiac output were made in 17 normal subjects (who were habitual smokers) using both low nicotine cigarettes fitted into a Silicajel filter and standard brand cigarettes. Small increases in cardiac output in three subjects after standard cigarettes, and five subjects after low nicotine cigarettes occurred; in one subject output decreased slightly after smoking a standard cigarette. The rest showed no significant change.

Skin temperatures were measured on the forehead, fingers, and toes by a U.N.A. clinical thermocouple. Generally, patients showed small drops in temperatures of the skin of fingers and toes (more marked in fingers) with low nicotine cigarettes and greater drops with ordinary cigarettes. The patients whose cardiac output increased after smoking had greater than average drops in skin temperature with low nicotine cigarettes.

In most cases heart rates increased with smoking, the changes being more marked with ordinary cigarettes. Large pulse rate increases were commonly, but not invariably, associated with increases in cardiac output.

Changes in skin temperatures were more marked in smaller patients and less pronounced in larger ones. The size of the patient had no noticeable relationship to rates or cardiac outputs.

113. ERYTHROMYCIN: OBSERVATIONS ON SERUM LEVELS AFTER ORAL ADMINISTRATION AND ON TREATMENT OF THIRTY-THREE PATIENTS

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(Introduced by Kenneth G. Kohlstaedt, M.D.)

Observations on the absorption of Erythromycin in healthy human subjects have shown that with doses of 400 to 600 mg. by mouth, serum levels will rise to a peak at about three hours. This peak exceeds 1 μg of Erythromycin per milliliter of serum in most subjects when the subjects are fasting. If the drug is taken by mouth in similar doses about an hour after eating, the levels are

much lower and rarely exceed 1 μg of Erythromycin per milliliter of serum. In patients receiving multiple doses of 300 mg. every six hours, about half of the levels exceed 1 μg. With multiple doses of 500 mg. every six hours, nearly all levels are above 1 μg, and three-fourths of the levels are above 2 μg.

Thirty-three patients with various infections have been treated. Excellent results were obtained in the treatment of infections caused by hemolytic streptococci and pneumococci. Apparently good results were obtained in a case of pharyngitis caused by *Staphlococcus aureus* and in a case of *Staphylococcus bacteremia*. One patient with subacute bacterial endocarditis caused by *Streptococcus salivarius* failed to respond satisfactorily to treatment with Erythromycin, and the organism became resistant. Two cases of cellulitis and one case of urethritis appeared to be cured. No beneficial effect was seen in two cases of *Pseudomonas aeruginosa* infection and in one case of *Proteus vulgaris* infection.

114. SOME EFFECTS OF THERAPEUTIC IRRADIATION ON STANDARD TESTS OF LIVER FUNCTION

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As part of a study of lipid transport in a variety of states including that resulting from exposure to ionizing radiations, the effect of therapeutic doses of radiation on total serum lipids and certain standard tests of liver function was studied in fifteen patients undergoing x-ray and radium therapy for cancer of the cervix uteri. Patients with this lesion were selected because of their relatively high energy absorptions, because the focus of irradiation was sufficiently remote from the liver to render unlikely an effect from direct radiation of this organ, and because patients could be selected whose disease had not yet produced serious systemic manifestations.

All patients had Stage I or II cancer of the cervix, and received similar courses of 24 daily x-ray exposures, succeeded by one hundred hours of radium implantation. Energy absorptions calculated by the method of Mayneord varied from 23 to 60 megagram-roentgens. No clinical evidence of radiation illness appeared. A battery of liver function tests including cephalin flocculation, thymol turbidity, thymol flocculation, zinc turbidity, bromsulfalein retention, and total serum lipids was performed before, during, and after treatment.

Changes during irradiation were encountered in bromsulfalein retention, cephalin flocculation, zinc turbidity, and total lipids.

Of the fifteen, seven showed unequivocal increases in BSP retention, three showed slight increases into the pathologic range, and five showed no alteration. When present, the increase in BSP retention appeared near the end of the course of therapy, and early in the post-therapy period.

Eight of the patients showed conspicuous decreases in total serum lipids which also appeared temporally related to the conclusion of therapeutic irradiation when total energy absorption was highest.

Changes in cephalin flocculation were less consistent, five of the fifteen exhibiting transitory positive reactions. Five of the fifteen also showed alterations in gamma globulin concentrations as measured by zinc turbidity, these changes tending to be an early increase succeeded by a later decrease.

The data suggest that transitory changes in BSP retention, cephalin flocculation, serum lipids, and gamma globulin concentrations may occur as an indirect result of therapeutic irradiation of carcinoma of the cervix uteri.

115. SERIAL OBSERVATION OF I^{131} UPTAKE BY THE THYROID FOLLOWING THYROTROPIN, CORTICOTROPIN, AND CORTISONE

CHARLES L. SPURR, M.D., FRANK J. KELLY, M.D. (BY INVITATION), AND HERBERT C. ALLEN, JR., M.D. (BY INVITATION), HOUSTON, TEXAS

Sensitivity of the scintillation counter permits repeated observations of thyroid I^{131} uptake using 1 μc doses. Effects of subcutaneous and intravenous thyrotropin (TSH) were assayed in 9 euthyroid subjects, 11 hypothyroid, and 8 patients with hypopituitarism. In euthyroid patients effects of intravenous corticotropin (ACTH) (3 cases), intramuscular corticotropin, and oral cortisone (3 cases) were similarly assayed.

Analysis of data indicates: (a) Euthyroid subjects receiving TSH (3.3 mg. every eight hours, total 10 mg. subcutaneously or 15 mg. intravenously twice a day for five days, total 150 mg.) demonstrated an increase in I^{131} uptake of about 100 per cent; (mean) six hours 123.6 per cent, twenty-four hours 99.3 per cent, forty-eight hours 104.9 per cent. (b) Certain hypothyroid patients were unresponsive, but those responding showed a per cent increase comparable to controls; (mean) six hours 90.5 per cent, twenty-four hours 74.5 per cent, forty-eight hours 107 per cent. (c) Hypopituitary patients demonstrated considerably greater increase in I^{131} uptake; (mean) six hours 323.4 per cent, twenty-four hours 735.2 per cent, forty-eight hours 767.2 per cent. The lower the initial uptake, the greater the response to TSH. As a group, euthyroid patients stimulated by subcutaneous TSH (10 mg.) returned to pretreatment levels within two weeks. Those receiving intravenous TSH (150 mg.) showed inhibition one and two weeks post treatment, but not at four weeks. (d) Intravenous ACTH (20 mg. daily) resulted in a reduction of I^{131} uptake to approximately half the control level; (mean) six hours 53 per cent, twenty-four hours 65 per cent, forty-eight hours 53.5 per cent. Intravenous ACTH inhibited I^{131} uptake to a degree comparable to the stimulatory influence of TSH in euthyroid subjects. One of three patients showed rebound activation one week after ACTH; six hours 92 per cent, twenty-four hours 42 per cent, forty-eight hours 44 per cent above control level; whereas the other patients remained 20 per cent below their control level. (e) Intramuscular ACTH, 10 to 20 mg. twice daily, cortisone 12.5 mg. twice daily, or both simultaneously, resulted in inhibition of I^{131} uptake, although not as uniformly as intravenous ACTH. Intramuscular ACTH resulted in greater inhibition than did cortisone in two of three subjects, and was comparable to intravenous ACTH in effect. One week following withdrawal of therapy, inhibition continued at 40 per cent of their control level; rebound was not observed. This group had been on continuous hormone therapy several weeks, whereas intravenous ACTH was given for four days.

116. FLUID EXCHANGE DURING INCIPIENT NEPHROGENIC HYPERTENSION IN DOGS

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(INTRODUCED BY LOIS N. KATZ, M.D.)

As a part of a series of studies on the pathogenesis of hypertension, an analysis was made of alterations in fluid exchanges in dogs following partial ligation of both renal arteries. Attention was focused on alterations in water intake and urine output during the period of *incipient hypertension,* i.e., during the time interval between the Goldblatt operation and the onset of hypertension.

Eight pairs of dogs were kept in metabolism cages for a seven- to fifteen-day control period. Surgery was then performed; bilateral renal artery constriction was done in one of the pair, sham operation in the other.

In 6 of 8 Goldblatt dogs, benign hypertension developed during postoperative days one to four, and persisted during subsequent weeks; none of the sham-operated animals exhibited a blood pressure rise.

In both experimental and control animals, anesthesia and surgery were followed by an increment in water intake and urine output. However, this increment was significantly greater in the dogs developing renal hypertension. This greater fluid exchange was present in the Goldblatt animals in the days both immediately prior to and immediately after the onset of hypertension. Further, throughout this period the dogs developing hypertension exhibited a greater increment in water intake (325 ml.) than in urine output (195 ml.).

These findings—that the development of renal hypertension in dogs is associated with an increased water exchange, and possibly with an occult H_2O retention—are further evidence of a relationship between body fluid and blood pressure regulation.

117. ALVEOLAR VENTILATION DURING THORACIC SURGERY

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(Introduced by Carleton B. Chapman, M.D.)

Acidosis during the course of anesthesia is well known, but the mechanism of its development has not been clearly defined in man. It has been thought by some to be respiratory and by others to be metabolic (due to the anesthetic agent) in origin.

Arterial blood pH and CO_2, total ventilation, and alveolar ventilation were studied in 13 patients before and throughout the period of intrathoracic surgery. In addition, a tracing was taken to show the rate and pattern of the artificial respiration administered by the anesthesiologist. The anesthetic was Pentothal-curare.

Results.—Prior to opening the chest there was only a slight fall in alveolar ventilation and rise in pCO_2. However, the alveolar ventilation fell abruptly when the chest was opened, despite an apparently adequate total ventilation (6 to 7 L. per minute). The mean alveolar ventilation fell from a control of 4 L. per minute to 2.14 L. per minute. In one subject it reached 0.850 L. per minute and the pCO_2 climbed to 117 mm. Hg in the presence of a total ventilation of 5.5 L. per minute. The drop in alveolar ventilation allowed a severe retention of CO_2 (highest pCO_2 117 mm. Hg, mean rise to 82 mm. Hg) while furnishing adequate oxygen for body needs. There was cyanosis in only one subject, and this was due to a temporary obstruction of the airway. Oxygen demands were met by diffusion because of the high oxygen content of the respired gas. The respiratory tracing revealed that the respiratory assistance was intermittent. Long pauses (up to 1 minute) occurred often as the anesthesiologist adjusted the intravenous infusion, administered blood, etc.

Conclusion.—CO_2 retention, due to an inadequate alveolar ventilation, is probably the most important factor in the severe acidosis which is commonly seen during thoracic surgery.

118. CHANGES IN THE JOINT TEMPERATURE AND POTENTIAL INDUCED BY INTRA-ARTICULAR COMPOUND F

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(INTRODUCED BY LOUIS R. LIMARZI, M.D.)

Previous work with rheumatoid arthritis patients has shown that, following administration of ACTH, bioelectric potentials of the knee joints fell within twenty-four hours from the pathologic level (30 to 60 millivolts) to the normal range (0 to 10 millivolts). Other investigators have found that within seventy-two hours joint temperatures decreased from 35° to 37° C. to the normal range of 33° to 34° C.

Responses to intra-articular administration of dehydro-cortisone acetate (Compound F) were studied by measurements of joint potential and joint temperature in a group of patients with rheumatoid arthritis. Temperature was determined by insertion of a thermocouple into the knee joint, using an 18-gauge needle. Immediately thereafter, a junction of isotonic NaCl was made with the synovial fluid. An isotonic salt junction was applied to the skin. By means of saturated KCl-calomel half cells, the difference of potential was determined. The statistical correlation between potential and temperature was determined for a group of 60 untreated rheumatoid arthritis patients. The mean joint temperature was approximately 36° C., as compared with a mean potential of about 45 millivolts. The potentials ranged from 25 to 60 millivolts, corresponding to a temperature range of 34° to 37° C. A positive correlation coefficient of 0.8 was determined.

Twelve rheumatoid arthritis patients were studied for a period of one week. Compound F (dehydrocortisone acetate), 25 mg. in isotonic NaCl, was injected into one joint cavity at weekly intervals for three or four weeks. Temperatures and potentials of both joints were determined four times weekly. In the treated joints, potentials fell rapidly from the mean initial level of 45 millivolts, reaching a minimum of 20 to 25 millivolts within twenty-four hours. By the end of the first week, the level had returned to about 35 millivolts. The responses during the subsequent weeks were similar. Temperatures in the treated joint responded more slowly, reaching, on the average, a minimum of about 34° C. on the third day, and subsequently returning toward the pretreatment level. In the untreated joint, the responses, though definite, were less rapid and of a smaller magnitude. The minimal potential, about 30 millivolts, and temperature, about 34.5° C., occurred between the third and sixth days.

119. INFLUENCE OF SOME EMULSIFYING AGENTS ON THE DIGESTIVE TRACT

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Despite the extensive use of emulsifying agents in recent years, little has been reported about their effect on various systems of the body.

In this study, therefore, the effects of two such agents—Span 60 and Tween 60—on the gastrointestinal tract are reported.

The first part of this study consisted of observing the effect of prolonged feeding of these substances on the digestive tract as elicited by changes in the (a) gas pattern of the bowel, (b) intestinal motility measured by the emptying of the stomach and movement of barium through the intestinal tract, and (c) function of the gall bladder.

The second part consisted in determining changes in gastric motility (balloon technique), gastric acidity, and bile flow following a single ingestion of 20 Gm. of the test substance. Any untoward symptoms following these substances were also recorded.

Ten patients who received Tween 60 (6 Gm. daily) for twenty-eight days showed no significant changes in the gas pattern of the bowel, in the emptying of the stomach, and in the appearance of the small bowel. Filling, visualization, and emptying of the gall bladder were also essentially unchanged. In four patients the head of the barium column was slightly more advanced. The results following twenty-eight days of Span 60 (6 Gm. daily) were essentially similar to those following Tween 60.

In six patients studied by the balloon technique, Tween 60 had little influence on gastric motility. Of five patients fed Span 60, two had a slight increase in gastric motility. There were no significant changes in the free acidity following either Tween 60 or Span 60. The effect on bile flow (through a T-tube) was equivocal, because of fluctuations in volume of bile (even during control period). No subjective symptoms were noted. The ingestion of Tween 60 or Span 60 did not produce any significant changes in the gastrointestinal tract measured by the above experimental setup in a small number of patients.

120. OBSERVATIONS ON A NEW ANTIAMEBIC SUBSTANCE

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Fumagillin, a new antibiotic, was recently introduced for the treatment of intestinal amebiasis. This report presents our experiences with this antibiotic in two separate groups of patients.

Fifteen cases of amebiasis (11 chronic and 4 acute) were treated in one hospital. Four patients were treated with 200 mg. daily for ten days. All developed anorexia, nausea, and epigastric distress; 2 developed maculopapular eruptions on the face and extremities; 2 developed an exfoliativelike dermatitis; and 1 developed psychoticlike symptoms on the sixth day. The stools of all 4 were free of ameba immediately at the end of therapy and have remained thus until now—three months later.

Eleven patients were treated with 100 mg. daily. These had moderate nausea with occasional vomiting. All had an occasional diarrheal stool after five days of treatment. Three patients developed macular eruptions on the face and extremities. All had negative stools at the end of therapy, but in 5 the stools became positive within three months. In all 15 cases there were no changes in urinalysis and liver profile studies.

In another hospital 25 cases were treated: Series 1 consisted of 5 "carriers," and they were treated with 150 mg. daily for ten days. Two patients had negative stools three months after cessation of treatment. Series 2 consisted of 10 "carriers." These patients were given 100 mg. daily for ten days. Stools of 7 patients were negative three months after cessation of treatment. Series 3 consisted of ten active cases. These patients were treated with 120 mg. daily for ten days. There were 4 failures out of 10 within a three-month period. No side reactions of note were present in any of these series.

These observations would suggest that fumagillin is an effective antiamebic substance, but that the larger doses (150 or more mg. daily) are associated with toxic symptoms.

121. CLINICAL STUDIES OF INTRAMUSCULAR INJECTION OF DIGITOXIN (DIGITALINE NATIVELLE) IN A NEW SOLVENT

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Digitoxin in alcoholic solution for intravenous use is commercially available, but the intravenous route may sometimes be impracticable. Digitoxin in an alcoholic solvent intended primarily for intravenous administration has been given intramuscularly on occasion, but it usually produces severe pain locally, and may produce tissue necrosis. To overcome these hazards, digitoxin has been prepared for intramuscular injection in a new nontoxic, nonirritating solvent, polyethelene glycol 300.

This solution was evaluated in the treatment of 25 patients with auricular fibrillation and congestive heart failure. Patients with auricular fibrillation were chosen because it was felt that the therapeutic effect of digitoxin could be more adequately evaluated in them, and that the observation of the ventricular rate furnished a valid criterion for the degree of digitalization.

Digitalis preparations were withheld in these patients until the ventricular rates became rapid and a pulse deficit developed. Usually this was associated with an increase in signs and symptoms of failure. Digitalization was then initiated by intramuscular injection of 1.2 mg. of digitoxin in 6 ml. of solvent, injecting 0.6 mg. into each gluteus maximus. The onset of ventricular slowing and the appearance of digitalis changes were determined by electrocardiograms at hourly intervals for five hours following injection. Slowing of the ventricular rate was evident in four hours.

Digitalization was completed and maintained for two to three weeks by daily intramuscular injections of digitoxin; the usual maintenance dose was 0.2 mg. daily. A very satisfactory therapeutic effect was obtained in all patients with minimal local and no undesirable systemic reactions. The effect of oral administration of digitoxin was compared under similar conditions in many of the group. The intramuscular preparation was found safe and effective in initiation and maintenance of digitalization.

122. CLINICAL AND ROENTGENOLOGIC EVALUATION OF BANTHINE IN THE COLON

MICHAEL H. STREICHER, M.S., M.D., AND WINIFRED WISE, B.S., CHICAGO, ILL.

This anticholinergic drug is methantheline bromide. Clinical studies indicate that the drug inhibits motility of the gastrointestinal tract. This is accomplished through an inhibition of the action of acetylcholine in the ganglia of the autonomic sympathetic and parasympathetic systems and in the postganglionic nerve endings of the parasympathetic system.

Our interest was directed primarily toward the effects of Banthine upon the motility of the colon. We have selected 50 patients and studied them clinically and roentgenologically. The group comprised 19 patients having constipation, 25 patients having ulcerative colitis, and 6 having spastic colitis. Normal controls were also included in this series.

Banthine was administered orally (50 mg. three times a day). Evaluation of the motility of the colon was made by roentgenologic study before and after administration of Banthine and by numerical records of daily bowel movements.

Daily records were maintained of the number of bowel evacuations before and after oral administration of Banthine.

On x-ray examination we concluded that in the 19 cases of constipation, 3 elicited hastened motility, 5 were unchanged as to time of evacuation, and 11 presented evidence of delayed motility.

Of 25 patients with ulcerative colitis, 2 presented evidence of hastened motility, 7 were unchanged, and 16 elicited delayed motility in evacuation of the colon.

The group of 6 patients with spastic colitis presented 2 cases of unchanged motility time, and 4 were delayed in evacuation time.

Of the 10 control (normals) patients we learned that after oral administration of Banthine, 2 patients were unchanged as to their motility time by roentgenologic study and 8 were delayed.

Clinical observations made of the entire group paralleled the findings made roentgenologically. Our conclusion thus far is that Banthine administered orally inhibits motility of the colon.

123. FOLD FORMATION OF THE ULTRASTRUCTURE MEMBRANE OF THE ERYTHROCYTE

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During a series of observations of the ultrastructure of normal and abnormal erythrocytes with the electron microscope, we encountered a case of iron deficiency anemia in which 90 per cent of the erythrocyte ultrastructure membranes showed thin smooth fold formation. This stimulated our interest in this variant, and further studies revealed that the folding phenomenon was present in 10 per cent and 50 per cent of the membrane population of two other cases of iron deficiency anemia. In 11 of 55 cases of chronic leukemia, folds were noted in 5 to 15 per cent of the membranes. One of the leukemias had 40 per cent of its membranes folded. Three of 43 specimens from normal individuals showed fold formation in 1 to 3 per cent of the membrane population.

It has previously been observed that fixatives as formalin and osmic acid could cause similar folding of the ultrastructure. In addition to these fixatives, other physical and chemical modifications resulting in folded membranes are copper sulfate, lead nitrate, ferric sulfate, mercuric chloride, and silver nitrate, lowering of the pH below 3.0, heavy sensitization and intense trypsinization.

The following hypothesis as to the folding mechanism is offered: during the process of hemolysis, osmosis causes some distention of the red cell; normally the ultrastructure is resilient, and there is contraction before drying is complete. If the elasticity is destroyed or is inherently diminished, there is failure of contraction, and the membranes fall together on the surface with the folds becoming visible. In the clinical observations the cells and membranes were not treated, so the fold formation is quite likely due to defective ultrastructure architecture.

124. THERAPEUTIC TRIAL OF AUREOMYCIN, CHLORAMPHENICOL, AND TERRAMYCIN IN MUMPS

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Preliminary reports of favorable effects of aureomycin, chloramphenicol, and terramycin therapy of mumps left the situation unclear. Since the disease is one of the most frequent specific viral infections in adults for which no therapeutic relief is available, a systematic therapeutic trial was carried out. A test procedure was used that allowed definite conclusions from few cases.

Six cases of mumps orchitis were treated with aureomycin (4 cases), chloramphenicol (1 case), or terramycin (1 case). Fever persisted for three days or more after the antibiotic was begun in all six cases. It was concluded that the antibiotics were without effect in the treatment of mumps.

The choice of mumps orchitis cases for treatment contributed largely to the significance of the results. The onset with testicular pain and fever was recorded with a high degree of reliability in patients' histories and sometimes by observation of the physician. The course was severe and predictable. Four of five cases had a total of three or more days of febrile course. Any regular alteration of the course by antibiotic therapy would be readily recognized during treatment of a small series of cases of mumps orchitis. The result was especially evident and required the smallest number of observed cases when, as in this study, the therapeutic agent had no effect upon the course of the disease.

125. ASSAY OF CREATINE IN BIOPSIED MUSCLE FROM CARDIAC PATIENTS

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We have previously reported the study of the concentration of creatine in the whole blood in normal individuals, various disease states, and in cardiac patients during and after recovery from failure. Blood creatine values were found to be highest in severe cardiac failure and to return to near normal values with compensation. Although muscle creatine values have been studied in patients dying of heart failure, there is no report of studies of biopsied human muscle. Skeletal muscle creatine content was determined in duplicate using one gram of muscle obtained by biopsy of the gastrocnemius muscle using local anesthesia of the skin.

Tissue creatine was studied in biopsy material from 28 patients during failure and before therapy. Muscle creatine showed a minimum of 195 mg. per cent, maximum of 395 mg. per cent, a mean of 251.

The blood creatine values taken at the time of the biopsy averaged 6.1 mg. per cent. In ten patients the biopsy was taken ten to fourteen days later when the patients were clinically compensated. The tissue creatine increased to a mean value of 329.8 mg. per cent, while the whole blood creatine values dropped to a mean value of 3.4 mg. per cent. A similar study was made of total blood creatine and tissue creatine before and after compensation in 10 patients who were given α tocopherol in addition to therapy identical to that given the preceding group. The whole blood level of this group before treatment averaged 6.17 mg. per cent. The initial muscle creatine level had a mean of 245.3 mg. per cent. Ten to fourteen days later after clinical evidence of compensation the tissue creatine level was 412 mg. per cent and the whole blood level was 3.4 mg. per cent.

126. PHYSICAL CHARACTERISTICS OF THE BALLISTOCARDIOGRAPHS RECORD AS RECORDED BY THE PORTABLE ELECTROMAGNETIC TYPE MACHINE

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Inexpensive construction and ease of use have brought increased interest in the ballistocardiogram as recorded by several types of portable instruments. The interpretation of the record produced by these simple machines is based, of necessity, upon the consideration that the character of the waves recorded by the newer portable machines and by the Stan. table (or other calibrated machines) are comparable. Accordingly, an analysis of the physical characteristics of ballistocardiographic record made by a portable electromagnetic machine was made of three groups of individuals. Three hundred fifty normal young men, approximately 150 individuals with normal cardiovascular systems but suffering from various other diseases, and 125 individuals with frank cardiac disease, were studied. The records were graded 0 through 4 on the method of Brown and DeLalla, the ratio of the K to the H wave calculated, the duration of time from the peak of H to depth of K measured, and the degree to which this time varied in successive cycles was noted.

The results of this study indicated the physical limits of the normal BCG recorded by this method. A mathematical comparison between the normal control group and the other two groups was made, and revealed remarkably small differences. Comparison of the BCG of normal individuals recorded by an electromagnetic type machine and by a calibrated table revealed definite differences in the K:H ratio. The former machine tends to record a deeper K stroke than the latter.

127. QUANTITATION OF VENOUS-ARTERIAL SHUNTS IN CONGENITAL HEART DISEASE ON THE BASIS OF DYE-DILUTION CURVES

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Use of the earpiece or cuvette oximeter makes it possible to record with relative ease the dilution pattern in arterial blood of T-1824 dye following rapid intravenous injection of the dye. In patients who have venous-arterial shunts, the dilution pattern is altered from the normal in that the time from injection to the first appearance of the dye is short, and in the majority of such cases an abnormal initial hump is apparent on the "build-up" slope of the main curve. The complete curve as recorded is the result of fusion of two separate components: (a) the dyed blood which has passed through the defect, proceeding by a short pathway to the recording instrument (shunt component), and (b) the dyed blood which has passed by the more circuitous normal route via the pulmonary vascular bed (normal component).

The assumption is made that uniform mixing of the dye has occurred before the division into "shunted" and "normal" components takes place. The relative volumes of blood comprising these two components can then be calculated by estimating the factors of passage time and mean concentration of dye from the initial segments of the dilution curves resulting from the "shunted" and

the "normal" blood. The resultant areas represent the quantity of dye passing through the defect and the dye passing through the normal circuit, respectively.

In 20 patients who had cyanotic congenital heart disease, the magnitude of the right-to-left shunt has been calculated on the basis of the dye curves and compared with determinations of shunt magnitude from data obtained by cardiac catheterization. The results by the two methods correlated well ($r = 0.89$), but a systematic deviation from the line of identity was found which was eliminated by use of an arbitrary correction factor. The standard deviation of the difference between the shunt values calculated by the dye method and from cardiac catheterization data was 7 per cent. It is suggested that the dye procedure offers promise as a relatively simple method for the estimation of the magnitude of the shunt in most cases of cyanotic congenital heart disease.

128. EVALUATION OF 1-HYDRAZINOPHTHALAZINE AS THE SOLE ANTIPRESSOR IN THE TREATMENT OF HYPERTENSIVE DISEASE

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A humoral pressor mechanism of central nervous origin may participate in the genesis of hypertension; since its action is antagonized by 1-hydrazinophthalazine (Apresoline, Ciba), this drug was tested in 97 patients. The majority were hospitalized for study and initiation of treatment, which, with appropriate controls, was continued for three to more than twelve months in those who showed favorable responses and for eight weeks before the drug was considered ineffective. The usual dose of 200 mg. four times a day was reached by slow increments; smaller doses sometimes were adequate in maintaining responses.

Patients were warned against side effects; these occurred in two-thirds of the group, persisted in 10, and were intolerable in 5. They were grouped as vascular (headache, edema, palpitation, angina pectoris) or extravascular (anorexia, nausea, vomiting, mild anemia, and a "grippy" state sometimes associated with fever and arthralgia).

Patients were classed as (a) essential hypertension, early, (b) severe, (c) malignant, (d) renal, (e) diencephalic, (f) "ancestral," and (g) sympathectomized. Severity was graded numerically to a maximum of 16 estimates of cardiac, renal, and cerebrovascular damage and the means of diastolic pressure. Responses were grouped from means of diastolic pressure as I (normal levels), II (less than 110 mm. Hg), III (ranging under 120 mm. Hg) and IV (no substantial change).

One-third of the patients fell in response Group I and another third in Group II; in these severity index decreased by about one-half. Improvement was most manifest in the cerebrovascular estimate; in the renal panel, progress of nephrosclerosis was checked, but its residues persisted. Because the cardiac status of some older patients deteriorated, this panel showed the least improvement; appropriate safeguards may obviate this disadvantage. Favorable responses were most common in those in whom the disease seemed to be neurogenic (diencephalic, "ancestral") and least common in those with advanced or primary renal disease. Response was otherwise independent of severity; it was not increased in those who had been sympathectomized.

In summary, 1-hydrazinophthalazine is an effective antipressor drug in a large proportion of hypertensive patients; favorable responses are associated with remission of many of the evidences of hypertensive vascular disease.

129. THE EFFECT OF BILATERAL ADRENALECTOMY ON PALLIATION IN METASTATIC CARCINOMA

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Bilateral adrenalectomy has been performed on 18 patients with far-advanced malignancies. These include 14 patients with carcinoma of the breast, 3 with carcinoma of the prostate, and 1 with carcinoma of the ovary. The majority of these patients had previously received treatment with surgery, steroids, castration and/or radiation, and had subsequently relapsed.

Of the 14 patients with carcinoma of the breast, there were 12 female and 2 male patients. Nine experienced marked subjective improvement as demonstrated by almost immediate relief of bone pain and increase in well-being, appetite, and body weight, and the reversal of debilitating status to an ambulatory and self-assisting condition. Of the 9 patients experiencing subjective improvement, major objective improvement has been demonstrated in five. This includes reduction in the size of the tumor masses, healing of cancerous ulceration, and recalcification of osteolytic lesions. In 2 patients experiencing subjective improvement, recurrences of pain developed in two and four months following adrenalectomy. Both of these patients showed evidence of progression of the malignant process. Three patients with carcinoma of the prostate have showed subjective improvement that has been sustained for two, four, and seven months. One of these patients also showed evidence of objective improvement. One other patient with carcinoma of the prostate experienced marked subjective improvement after unilateral adrenalectomy but relapsed after freedom from pain for five months. No change in the course of the disease was noted following adrenalectomy in the one patient with carcinoma of the ovary.

Hypocorticalism has been adequately controlled on all patients with a maintenance dose of 50 mg. of cortisone acetate by mouth daily. Larger doses have been necessary in periods of stress.

No patient showing objective improvement has relapsed to date, the longest period of observation being eight months.

We have been unable to predict which patients can be expected to respond to this form of therapy. Roentgenograms and kodachromes will be presented to demonstrate objective improvement in these patients.

130. FURTHER STUDIES ON THE CARDIAC LESION OF THE GENERALIZED SHWARTZMAN REACTION IN RABBITS INFECTED BY GROUP A STREPTOCOCCI: NECROTIZING ARTERITIS WITH FIBRINOID DEPOSITS IN VESSEL WALLS

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In the generalized Shwartzman reaction produced by two intravenous injections of meningococcal toxin, areas of acute myofiber necrosis were encountered in the hearts of some rabbits. Lesions of the blood vessel walls were not observed in such animals.

When an intravenous injection of living group A streptococci was substituted for the first injection, and the provoking injection of meningococcal toxin was given forty-eight hours later, a new type of cardiac lesion occurred which conspicuously involved the arteries of the heart. Extensive deposits of

fibrinoid material appeared within the arterial walls within twenty-four to forty-eight hours after the injection of toxin. The fibrinoid was sometimes thickly layered beneath the endothelium; in some segments it occupied the entire wall and obliterated all normal structural elements. Accumulations of fibrinoid also appeared in perivascular spaces. Necrosis of the arterial wall occurred in segments containing dense fibrinoid deposits, sometimes with rupture of the wall. Varying degrees of inflammatory reaction by "owl-eyed" and multinucleated cells were seen around the involved vessels.

Such arterial lesions were not demonstrable in organs other than the heart. Bilateral cortical necrosis of the kidneys, associated with the occlusion of glomerular capillaries by fibrinoid material, occurred in 47 per cent of animals with myocardial arteritis.

The incidence of the arterial lesion was doubled in rabbits which had had a previous streptococcal infection one to two months before the experiment. For example, in a group of 37 animals given a single injection of streptococci followed by toxin two days later, myocardial arteritis occurred in 27 per cent. In 47 rabbits which had undergone a previous infection, the same procedure resulted in arterial lesions in 55 per cent. The mechanism underlying this enhancement is under study.

The absence of these arterial lesions in the conventional generalized Shwartzman reaction, produced without streptococci, suggests that a systemic streptococcal infection may involve, in the rabbit, a special effect on the blood vessels of the heart.

131. THE LACK OF INFLUENCE OF PENICILLIN ON THE COAGULATION OF THE BLOOD

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(INTRODUCED BY JOHN S. HIRSCHBOECK, M.D.)

Since Moldawsky, Hasselbrock, and Cateno suggested, in 1945, that penicillin had an effect on blood coagulation, reports on this subject have not been in agreement. This study was undertaken to determine whether or not alleged effects of penicillin on blood coagulation were of sufficient magnitude to deserve clinical consideration. Experiments were done in vitro and on human subjects on the effect of penicillin on the prothrombin time, coagulation time, thrombin activity, prothrombin consumption, labile factor, clot retraction, and protamine titration of normal blood. It was shown for the first time that penicillin did not affect prothrombin consumption, labile factor, thrombin activity, and the prothrombin titration. In addition, work that had failed to show an effect of penicillin on the coagulation time, prothrombin time, and clot retraction was confirmed.

Thus it seems likely that certain effects on blood coagulation that have been attributed to penicillin may have been due to impurities present in early preparations of this drug. Our conclusion is that at the present time the possibility of an effect on blood coagulation need not be considered when the pure crystalline penicillin is used.

132. LUNG BIOPSY IN DIFFUSE PULMONARY LESIONS

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We, in the field of pulmonary diseases, are unable to arrive at a diagnosis in certain cases of diffuse lung disease by usual methods of investigation. Differentiation is often dependent upon adequate tissue for histopathologic, bacteriologic, and chemical analytic studies. We have felt that a biopsy of a sufficient amount of lung tissue in such cases was indicated for one or more of the following reasons: (a) diagnosis, (b) prognosis, (c) medico-legal purpose, (d) improvement of our knowledge of disseminated linear and nodular lung infiltrations in life, and (e) evaluation of response to treatment.

In the last twenty-seven months 35 lung biopsies in 34 patients were obtained by a simplified technique of intercostal thoracotomy without rib resection perfected by one of us (D.B.E.). By this method, up to 10 Gm. of lung tissue has been obtained. The second biopsy in one patient (with chronic beryllium poisoning) was performed to evaluate the response to steroid therapy. One death resulted (case of disabling asbestosis) in the form of an acute myocardial infarction. Otherwise no complications nor significant morbidity ensued. The average hospital stay for the biopsy was four and one-half days.

The following diagnoses were obtained for the 34 patients.

Neoplasms—10
- Primary
  - Benign—1 (leiomyoma)
  - Malignant—1 (multifocal nonsecretory bronchiologenic adenocarcinoma)
- Secondary—7 (including one patient with soft part sarcoma)
- Unclassified as to primary or metastatic—1

Chronic interstitial pneumonitis—8
Occupational disease—5
- Chronic berylliosis—4
- Asbestosis—1

Sarcoidosis—5
Fungus—3 (each "benign" precalcified histoplasmosis)
Miscellaneous—3
- Pulmonary eosinophilosis
- Lipoid pneumonia (endogenous)
- Miliary tuberculosis (healing phase)

Each of the patients presented certain interesting clinical features. The resulting diagnoses had not been considered in 23 of the 34 cases. A prior poor prognosis was altered by the findings in 11 cases. Conversely, 5 patients found to have diffuse malignancy were asymptomatic. The management and treatment were especially aided in 22 of the 34. Although there was a confirmation of occupational etiology in 5 patients, it was disproved by biopsy in 3 others.

For the indications outlined, and when the diagnosis is otherwise unobtainable, we have felt that the results of surgical lung biopsy have justified the procedure.

133. A CASE OF SEVERE PROTEUS BACTEREMIA TREATED SUCCESSFULLY WITH LARGE DOSES OF PENICILLIN

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(Introduced by Francis D. Murphy, M.D.)

The relative resistance of Proteus organisms to most of the antibiotics makes it likely that infections due to this species will increase. This report is of a case

of severe Proteus bacteremia in which all of the available antibiotics were tried before cure was effected by the use of twelve grams per day of penicillin.

The patient was a 42-year-old chronic alcoholic who developed a high spiking fever and the clinical picture of bacteremia due to gram-negative bacilli other than the Salmonella. During his illness Proteus organisms were grown from his blood on nine occasions. The organism was shown in vitro to be moderately sensitive to penicillin, chloramphenicol, neomycin, and streptomycin. These drugs were used singly and in combination without any evidence of clinical response. Cure finally was effected when 12 Gm. per day of penicillin was given by the intravenous route for six weeks.

This case is considered to merit publication as a single case report because it illustrates the clinical effectiveness of penicillin against Proteus organisms, the essential clinical features of bacteremia due to gram-negative bacilli, and the clinical use of the tube dilution method of determining bacterial sensitivity to antibiotics.

In addition, in vitro studies with the Proteus isolated from the blood of this patient revealed that the combination of penicillin and chloramphenicol resulted in diminished bactericidal activity, but at the same time a potentiation of the bacteriostatic activity occurred. These results were confirmed using many other strains of Proteus, and their implications on the clinical use of combinations of antibiotics will be discussed.

134. THE EFFECT OF PROLONGED FEEDING OF LARGE AMOUNTS OF TWEEN 60 AND SPAN 60 TO HUMAN BEINGS

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(INTRODUCED BY HANS POPPER, M.D.)

To study the toxicity of the emulsifiers Span 60 and Tween 60 used in food and medications, the clinical picture and hepatic, renal, and hematologic functions were studied in inmates of an old people's and chronic disease infirmary (screened for absence of systemic diseases) who were fed for twenty-eight days 6 Gm. of Tween 60 (19 persons), 6 Gm. of Span 60 (16 persons), and 6 Gm. of corn oil (9 persons), respectively. Before, during, and following the treatment, the following were determined in addition to urinalysis: in serum, total protein, albumin, globulin, cephalin flocculation, thymol turbidity, zinc sulfate, phenol, gamma globulin turbidity, bilirubin, bromsulfonphthalein retention (5 mg. per kilogram within forty-five minutes), NPN, creatinine, total cholesterol, cholesterol esters; in blood, urea nitrogen, hemoglobin, white cell, red cell, and differential count as well as red cell fragility.

The medication produced no clinical untoward reactions. No alterations of the laboratory findings occurred with the following exceptions: variations in zinc sulfate, gamma globulin, and phenol turbidity occurred in a significant percentage without relation to the type or period of medication. Caution is recommended in the interpretation of a single abnormal result in these tests. Slight increase in BSP retention (not exceeding 12 per cent) occurred in few subjects of the initial group, again not related to period of medication; therefore, 15 additional patients of the infirmary and 10 young volunteer physicians each were fed, for twenty-eight days, 6 Gm. Tween 60, 6 Gm. Span 60, and 6 Gm. corn oil, respectively. In these 77 subjects the BSP retention as well as serum phospholipids was determined. Also, in these groups no clinical reactions occurred; the variations in BSP retention found in the previous series were not reproduced and the serum phospholipids did not change. The BSP

retention found in the first series was therefore not attributed to the medication but to the use of a relatively large load of BSP which seems to produce occasionally abnormal findings, especially in older people.

From the available data it appeared that the feeding of up to 6 Gm. of Span 60 or Tween 60 daily for twenty-eight days is without deleterious effect to human beings.

135. COMPARATIVE EFFECTS OF HYDROCORTISONE, HYDROCORTISONE ACETATE, AND CORTISONE ACETATE IN RHEUMATOID ARTHRITIS

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Rochester, Minn.

Hydrocortisone or one of its esters, administered orally or intramuscularly to 27 patients who had rheumatoid arthritis, produced antirheumatic effects similar in nature to those resulting from administration of cortisone to the same patients. One patient has maintained 90 per cent relief of rheumatoid manifestations during one year of continuous oral administration of hydrocortisone or hydrocortisone acetate.

When administered intramuscularly, hydrocortisone acetate and hydrocortisone cyclopentylpropionate appeared to be absorbed more slowly than cortisone acetate, as judged by clinical observations and by changes in erythrocytic sedimentation rate and in urinary excretion of nitrogen, 17-ketosteroids, and corticosteroids. Hydrocortisone acetate in daily intramuscular doses of 100 mg. or less generally appeared to produce slightly less antirheumatic effect than similar doses of cortisone acetate; however, the relatively short duration of the studies in which intramuscular administration was employed may have weighted the evidence in favor of the more rapidly acting cortisone acetate.

The antirheumatic potencies of orally administered hydrocortisone, hydrocortisone acetate, and cortisone acetate were compared, milligram for milligram. Hydrocortisone appeared to be somewhat more effective than cortisone acetate, since essentially equal antirheumatic effects generally were obtained by use of about 15 to 35 per cent less hydrocortisone than cortisone acetate. However, a significant portion of this difference can be accounted for by the fact that cortisone comprises by weight only 89 per cent of a molecule of cortisone acetate, the remainder being the acetate radical. Hydrocortisone acetate was less effective than cortisone acetate or hydrocortisone; from 25 to 100 per cent more hydrocortisone acetate was required to produce an antirheumatic effect equal to that of hydrocortisone. Differences in solubility and absorption probably account for some of the quantitative differences in effects of these three compounds.

Hypercorticism of essentially similar nature resulted from the administration to the same patients of excessive doses of hydrocortisone, hydrocortisone acetate, or cortisone acetate. In the doses used during these studies of prolonged oral administration, none of these three compounds appeared appreciably superior to the others in prevention of the occurrence of clinical signs and symptoms of hypercorticism.

136. RENAL CALCULI OCCURRING AS A COMPLICATION OF LYMPHOMA

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Elevation of serum uric acid and increased urinary excretion is known to occur in leukemia. Since uric acid is the chief end product of purine metabolism, increased formation may be expected whenever excessive breakdown of nucleoproteins occurs. With the advent of potent therapeutic agents for lymphomas, there have been occasional reports of uremia due to uric acid obstruction of the urinary tract. The occurrence within a short period of time of two fatal cases of uremia due to urate deposits and uric acid calculi prompted an investigation into the incidence of these complications in patients with lymphoma. Accordingly 186 consecutive admissions with diagnoses of acute or chronic leukemia, lymphosarcoma, reticulum-cell sarcoma, agnogenic myeloid metaplasia or giant follicular lymphoblastoma were surveyed and the incidence of uric acid calculi determined. The incidence was compared with the incidence in 100 cases of other types of metastatic malignancies.

The incidence of uric acid calculi in the lymphomas was 7.04 per cent. No uric acid calculi were found in other treated malignancies. The incidence of uric acid calculi in general hospital admissions is 0.07 per cent. The frequency of uric acid calculi in lymphomas is therefore 100 times as great as that occurring in general hospital admissions.

One fatality occurred in a patient treated with nitrogen mustard in whom, despite rapid regression of the lymphoma, death resulted from obstructive uremia. Another fatality occurred during spontaneous regression of the liver and spleen in myeloid metaplasia with subsequent uric acid obstruction despite repeated cystoscopic manipulation.

This potentially fatal and frequently avoidable complication of lymphomas is more common than has been realized. With extensive destruction of nucleoproteins by potent therapeutic agents, the probability of uric acid obstruction is increased. Therefore, adequate precautions against renal complications should be instituted as a part of the therapeutic regime. Administration of alkali, maintenance of adequate urinary output, evaluation of kidney function, and repeated determinations of serum uric acid and nitrogen retention, as well as frequent examination of the urinary sediment, are indicated.

137. CONCENTRATION OF ANTIBIOTICS IN THE BRAIN

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(INTRODUCED BY WALLACE E. HERRELL, M.D.)

It has been suggested that neopenil (the diethylaminoethyl ester hydriodide salt of penicillin G), a new form of penicillin for parenteral use, is concentrated in the human lung and in the brain of experimental animals. The following study was undertaken in an attempt to determine the concentrations of this, as well as several other antibiotic agents, in the human brain.

The patients studied in this investigation had been hospitalized because of psychiatric disorders at the Rochester State Hospital. Performance of prefrontal lobotomy had been scheduled for therapeutic reasons. Prior to operation, these patients received for prophylaxis one of the following drugs: neopenil, procaine penicillin G in aqueous suspension, aureomycin, terramycin,

dihydrostreptomycin, or erythromycin. At the time of operation blood, cisternal fluid, cerebrospinal fluid from the lumbar area and brain tissue were collected for antibiotic assay.

After the administration of neopenil, laboratory determinations revealed that significant concentrations of this agent occurred consistently in the serum, cerebrospinal fluid, and brain tissue. On the other hand, assay after the administration of procaine penicillin G in aqueous suspension disclosed significant concentrations of this agent in the serum, but none was detectable in the cerebrospinal fluid or brain tissue. Dihydrostreptomycin was not found in the brain, but significant amounts were reported in the serum and cerebrospinal fluid. Aureomycin, like neopenil, was found consistently in the serum and brain tissue. Although it has been established that aureomycin crosses the blood-brain barrier into the cerebrospinal fluid, none was found in this study. This may be explained by the fact that the cerebrospinal fluid was not refrigerated immediately. Erythromycin and terramycin were found to be similar in that, in most instances, only small amounts or none were found in the cerebrospinal fluid or brain.

The data presented in this study indicate that of the antibiotic agents investigated, only neopenil and aureomycin can be detected consistently in significant amounts in the brain tissue, after they have been administered. These findings may be of some significance in connection with the use of various antibiotic agents in the treatment of infections of the central nervous system.

138. ABNORMALITIES OF CARDIAC ACTIVITY STUDIED BY THE CAPACITANCE METHOD

WILLIAM V. WHITEHORN, M.D., CHICAGO, ILL.

Changes in the capacity of a condenser whose field includes the precordial thorax are produced by cardiac activity and can be appropriately detected and recorded. (Whitehorn and Perl: *Science* **109**: 262, 1949.) Such "cardiodielectrograms" (CDG) exhibit a characteristic pattern resembling classical ventricular volume curves, and are strikingly similar to records of ventricular border movements and densograms recorded with the electrokymograph. Evidence suggests that positional and volumetric changes in the thorax secondary to mechanical cardiac events are primary factors in production of the capacitance changes.

Studies on more than 100 subjects have established normal patterns and defined relationships between portions of the capacitance curve and events of the cardiac cycle. These form the basis for comparison with records from 150 outpatients with cardiac disease. Abnormalities of contour, amplitude, and timing are frequent. Examples of abnormal records in valvular, hypertensive, arteriosclerotic, and coronary cardiovascular disease will be presented and considered in relation to reported findings by Eky, and other methods in similar conditions. Advantages, disadvantages, and potentialities of the capacitance method for the study of human cardiac activity will be discussed.

139. THE SYNDROME OF VASCULAR COLLAPSE DUE TO GRAM-NEGATIVE BACTERIA: ITS MANAGEMENT WITH L-NOREPINEPHRINE AND ANTIBIOTICS

ROBERT I. WISE, M.D. (BY INVITATION), JAMES M. SHAFFER, M.D. (BY INVITATION), AND WESLEY W. SPINK, M.D., MINNEAPOLIS, MIN.

Shock resulting from acute infections associated with the polysaccharides of gram-negative bacilli is a serious clinical problem which has been little recog-

nized and poorly understood. Its seriousness is amplified by the failure of the shock to respond when treated by methods which are usually used in the management of hypotensive states. It is the purpose of this report to outline the results of the management of a series of cases with shock resulting from gram-negative bacteremia in which the blood pressure was supported by l-norepinephrine. Such treatment provided sufficient time to institute antibiotic therapy aimed at the underlying infection.

During the sixteen months from March, 1951, to July, 1952, 53 patients with positive blood cultures due to gram-negative bacilli were studied at the University of Minnesota Hospitals. In 8 of these cases the bacteremia was accompanied by severe hypotension and shock, and the etiologic agents were *Aerobactor aerogenes* in 3, *Escherichia coli* in 2, *Proteus mirabilis* in 1, Paracolon in 1, and *Salmonella typhimurium* in 1. Portals of entry were the gastrointestinal tract, genitourinary tract, biliary tract, and through an intravenous infusion. The clinical picture was characterized by restlessness, fever with or without chills, severe hypotension followed by a comatose state, and usually accompanied by a neutrophilic leukocytosis. Norepinephrine was administered by intravenous infusions in amounts necessary to maintain a normal blood pressure. The largest total dosage required was 532 mg., and the largest single daily dose was 136 mg. The longest duration of norepinephrine therapy was twenty-one days. It was demonstrated that with l-norepinephrine the blood pressure could be promptly elevated and maintained, which permitted the use of antibiotic therapy. Although 6 of the 8 patients ultimately succumbed, the periods of survival were longer than anticipated, and the recovery of 2 patients could be attributed to the use of l-norepinephrine. The results of this study indicate that l-norepinephrine is a useful adjunct in the management of the serious hypotensive states associated with gram-negative bacteremia. The potential hazards associated with the prolonged administration of large amounts of l-norepinephrine will be discussed.

140. THE HEMOLYTIC COMPONENT OF POSTIRRADIATION ANEMIA AS STUDIED WITH A MODIFIED ASHBY TECHNIQUE IN THE RABBIT

CLAUDE-STARR WRIGHT, M.D., MATTHEW C. DODD, PH.D. (BY INVITATION), BERTHA A. BOURONCLE, M.D. (BY INVITATION), AND JOSEPH L. MORTON, M.D. (BY INVITATION), COLUMBUS, OHIO

Considerable evidence has suggested a hemolytic component in postirradiation anemia. The necessity for using intense whole-body irradiation for investigating this "potential" hemolysis required the development of a suitable technique in the experimental animal. A procedure was devised for the study of red blood cell survival in rabbits by the use of a modified Ashby technique. Donor and recipient bloods were separated after transfusion by differential agglutination using isoimmune rabbit sera.

The study of red blood cell survival following intense irradiation of *recipient* rabbits was unsatisfactory. This is probably explained by the recent observations (Furth and associates) on the marked vascular damage and escape of red blood cells into the lymphatic system after heavy whole-body irradiation. For this reason the procedure was modified and survival was studied in 12 normal recipient rabbits transfused with the blood of 12 *donors* which had received 850 r total-body irradiation four days previously.

Results of these survivals compared with 21 normal control survivals were statistically treated, and indicate a significantly shorter survival for irradiated red blood cells.

141. THE DEGREE OF CORONARY ATHEROSCLEROSIS IN BILATERALLY OOPHORECTOMIZED WOMEN

JOHN H. WUEST, M.D. (BY INVITATION), AND JESSE E. EDWARDS, M.D. (BY INVITATION), ROCHESTER, MINN.

Common acceptance of the observation that there is a marked disparity in the average degree of coronary atherosclerosis between men and women of comparable ages has prompted the study of the degree of coronary atherosclerosis in castrated women.

In a study of cases of bilaterally oophorectomized women who had come to necropsy, 49 cases fulfilled the criteria acceptable for this study. The criteria were (a) an interval of at least two years between oophorectomy and death, (b) menopause not occurring prior to oophorectomy, and (c) age at oophorectomy less than 45 years, regardless of menstrual history. In each case, the heart was available for study regarding the state of the coronary arteries.

The degree of coronary atherosclerosis in each decade was greater than in a control series of 600 hearts of women studied by Ackerman and his associates, but it was less than in a control series of 600 hearts of men studied previously by White and his associates.

It has been reported previously that the livers of men with cirrhosis fail to inactivate normally-occurring estrogens. A preliminary study of the degree of atherosclerosis in the coronary arteries of 59 men with cirrhosis of the liver compared with control studies by Ackerman and his associates and by White and his associates disclosed a lower degree of atherosclerosis as compared to the control study on men and at some ages lower than the values in women.

It appears that estrogens may play a role in the complicated metabolic matrix representing the pathogenesis of atherosclerosis.

142. ANIMAL AND HUMAN STUDIES ON THE TOXICITY OF POLYMYXIN B WITH PARTICULAR REFERENCE TO AN EVALUATION OF RENAL FUNCTION

ELLARD M. YOW, M.D. (BY INVITATION), AND JOHN H. MOYER, M.D., HOUSTON, TEXAS

Because of the effectiveness of Polymyxin in some infections due to bacteria resistant to all other antibiotics, it seemed important to evaluate further its toxicity. The B fraction was selected for this study.

Polymyxin B was administered intramuscularly to 26 dogs and 40 patients in doses varying from 1.5 to 5 mg. per kilogram per day for periods varying from one to eight weeks. Control and weekly differential renal function tests were performed in all the dogs and in ten of the human subjects. These included glomerular filtration rate, maximum tubular function, renal blood flow, creatinine clearance, and sodium and potassium excretion. In all the patients the usual clinical renal function tests were routinely followed, as well as observation for other signs of toxicity. At the end of the animal experiments, sections of the heart, liver, and kidney were examined histologically.

There was no decrease in renal function in the dogs when given 2.5 mg. per kilogram, but when the dose was increased to 5 mg. there was a decrease in both GFR and TM_G. In five human subjects, with no evidence of functional renal disease, the daily intramuscular injection of 2.5 mg. per kilogram produced no changes in the GFR and only a slight decrease in the TM_{PAH} (92 per cent of

control value). There were no significant changes in creatinine clearance. In five patients in whom there was evidence of chronic renal insufficiency, the average GFR was reduced to 85 per cent of control values and the TM_{PAH} to 62 per cent. The creatinine clearance and renal blood flow were likewise decreased in this group. In the remaining patients in whom clinical function tests were performed, no significant changes were observed.

The histologic study of the kidneys of dogs receiving Polymyxin revealed evidence of tubular degeneration in those showing the most marked functional changes.

It may be concluded from this study that Polymyxin B may be safely given intramuscularly in doses of 2.5 mg. per kilogram per day in patients with relatively normal kidneys. Smaller doses should be used in patients with chronic renal insufficiency, 2.0 mg. per kilogram or less depending on the degree of renal damage. No other serious side reactions were observed.

143. COLD AGGLUTINATION OF SHEEP ERYTHROCYTES AS A FACTOR IN FALSE-POSITIVE HETEROPHILE AGGLUTINATION TESTS

C. J. D. Zarafonetis, M.D., H. L. Oster, M.D. (By Invitation), and Virginia F. Colville, B.S. (By Invitation), Philadelphia, Pa.

Experience in this laboratory indicates that certain discrepancies encountered in the results of heterophile agglutination tests may be due to factors not generally appreciated. For example, the titer of a given serum specimen may vary widely in tests with erythrocytes from different sheep. The present discussion is concerned with still another factor, namely, the cold agglutinin phenomenon as a possible source of error in the heterophile agglutination test.

Results of a study involving heterophile agglutination tests on 6,432 serum specimens from 2,221 patients form the basis of this report. The patients represent a wide range of clinical material, including infectious mononucleosis and many other disorders. The technique of test employed has been previously described. It is sufficient to indicate, therefore, that it provides for readings of the agglutination titers after the refrigeration phase of the test, and also after appropriate water-bath incubation (37.5° C.) to dispel any cold agglutinin effects which may be present.

The pertinent findings of this study and their implications may be summarized as follows: (a) Cold agglutinins for sheep erythrocytes may be present in sera from patients with infectious mononucleosis and other disorders. (Examples of cold agglutination in tests with sera from cases of leukemia, lymphoma, various anemias, collagen disorders, and acute and chronic infections are included.) (b) The cold agglutinin titer for a given serum specimen may vary widely in tests with erythrocytes from different sheep. (c) As a corollary, erythrocytes from one sheep may give high cold agglutinin titers with the test sera, while cells from another sheep may agglutinate poorly or not at all in similar tests with the same sera. (d) Since many laboratories record titers of heterophile agglutination tests directly after overnight refrigeration of the test, it seems reasonable to conclude that many so-called "false-positive heterophile agglutination" results are due to the phenomenon of cold agglutination. (e) It is possible to eliminate the cold agglutinin factor by simply re-warming the test serum-cell mixtures following overnight refrigeration.

144. SEROLOGIC TESTS FOR SYPHILIS IN INFECTIOUS MONONUCLEOSIS

C. J. D. Zarafonetis, M.D., and John F. Kent, Ph.D. (By Invitation), Philadelphia, Pa.

Reports vary widely as to the incidence of "false-positive" serologic tests for syphilis in infectious mononucleosis. The present report deals with the results of 8 different tests for syphilis on 594 sera from 183 patients with infectious mononucleosis. Thirteen (7.1 per cent) of the patients had sera which gave positive or doubtful results in 1 or more of the tests employed. The case incidence of false-positive reactions was only 1.1 per cent in each of the 4 microflocculation tests (Venereal Disease Research Laboratory, Kline cardiolipin, Rein-Bossak cardiolipin, and Mazzini), and in the EP50 complement fixation test. The Kahn standard test was positive in 2.2 per cent of cases, and doubtful in an additional 3.3 per cent. The Kolmer standard and cardiolipin complement fixation procedures were positive in 2.2 per cent of the subjects. Not one of the serum specimens was positive in all 8 tests or even in all three types of tests.

The relatively low incidence of positive reactions and the temporal vagaries of sampling did not permit an extensive analysis in regard to the duration of the false-positive reactions with each of the respective tests. From the available data, however, it appears that when the complement fixation tests were positive, they tended to be positive earlier in the course of illness, and to become negative sooner than did the Kahn test. In one instance, the complement fixation tests also reverted to negative before the microflocculation tests.

It appears that both the incidence and the duration of false-positive reactions for syphilis are dependent to a considerable degree on the test method employed with sera obtained from patients with infectious mononucleosis.

145. ACTION OF ISONICOTINIC ACID HYDRAZIDE AND RELATED COMPOUNDS ON ENZYMES OF BRAIN AND OTHER TISSUES

E. A. Zeller, M.D., Ph.D., J. Barsky, M.A., E. R. Berman, M.S., and J. R. Fouts, B.S., Chicago, Ill.

(Introduced by S. Freeman, M.D., Ph.D.)

Various effects of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazine (IIH) on the central and autonomic nervous system have been reported. The question thus arose as to whether well-defined enzyme systems of the nervous system are affected by the new antituberculous drugs. Previously, it has been shown (*Experientia* **7**: 349, 1952) that the two compounds, like all other basic antitubercular drugs and antibiotics tested, inhibit diamine oxidase of mycobacteria (*J. Bact.* **62**: 53, 1951; *Federation Proc.* **11**: 316, 1952). While neomycin, viomycin, and streptomycin have little effect on mammalian diamine oxidase ("histaminase") the two hydrazides inhibit the bacterial *and* the mammalian enzyme. Monoamine oxidase, an important agent for the inactivation of epinephrine, was prepared by the isolation of the mitochondria of the brains and livers of six different species, including that of the human brain. In all instances, monoamine oxidase was very sensitive to 0.1 millimolar IIH, while 1.0 millimolar INH caused a marked depression of enzyme activity only in cat and mouse brain mitochondria. After injection of 10 to 20 mg. of IIH into adult male and female rats, the animals were sacrificed

and liver and brain mitochondria isolated. Monoamine oxidase activity disappeared almost completely. INH had no effect.

The two types of cholinesterase from human brain and from other sources are not influenced by the two hydrazides.

In addition to these compounds, two isomers of INH, nicotinic acid hydrazide and picolinic acid hydrazide, were used in similar experiments as described above. Their action on monoamine oxidase was much less marked than the one given by IIH. Again cholinesterases and ali-esterase were not sensitive. But picolinic acid hydrazide was found to be one of the strongest inhibitors of mammalian diamine oxidase ever found. Its action on the whole animal will be described.

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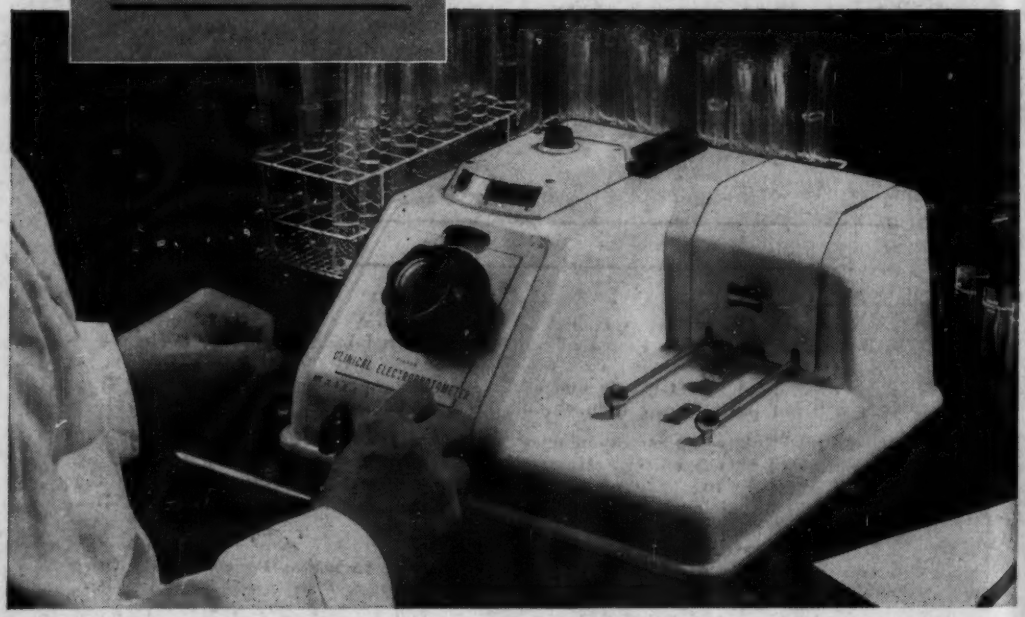

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